

The TCS CellWorks AngioKit

A Human Multicellular in vitro Model of Angiogenesis

The TCS AngioKit model accurately reproduces the different phases of the angiogenesis process using a co-culture of human endothelial cells with other human cell types in a specially developed medium. Capillary development in the model can be conveniently followed by fixing the cultures and staining with antibody to von Willebrand factor or PECAM-1. The endothelial cells start by forming small islands in a monolayer of matrix cells (Fig 1a). The endothelial cells begin to proliferate, then enter a migratory phase where they can be seen to move within the background monolayer to form thread-like, tubular structures by day 7 (Fig 1b). By approximately day 12 to 14, these structures gradually join up to form an intricate network of anastomosing tubules resembling the capillary bed of the chick chorioallantoic membrane (Fig 1c). The “vessels” formed by this process can be seen to originate from the islands of endothelial cells formed during the proliferative phase. By day 14 the tubules are wider and thicker with patent lumina containing coated pits and caveolae and with tight junctions between interdigitating cells of the vessel walls (Bishop *et al*, 2000)

Tubules can also be visualised by staining for Collagen IV, a constitutive element of the extracellular matrix in neovascular tissue (Fig 2). The tubules produced also stain positive for the endothelial cell adhesion molecule PECAM-1 (Fig 3) and for ICAM-2 (not shown).

Tubule formation can be assessed using image analysis and quantified using the TCS ‘AngioSys’ software package. In summary, stained tubules captured by the analysis software are reduced in width to a single pixel. The total number of pixels in a given field of view therefore represents the length of tubules. Subsequently, means of tubule length, standard deviations and coefficients of variation can be calculated. Total vessel number, total tubule area and the number of branch points formed can be similarly analysed.

Test runs with the assay have shown it to be reproducible and robust when operated in 24-well plates. Using the analysis methods described above to score tubule formation, the observed coefficient of variation between assays is of the order of 10%.

The AngioKit provides a model in which human endothelial cell-derived tubules develop slowly over a time course (typically 10- 12 days) which permits the evaluation of both potential angiogenesis inhibitors and activators. No other practical model permits studies of this sort. (Donovan *et al*, 2001). The co-culture model is set up in 24-well culture dishes and so is suitable for screening studies.

Test Substances

A wide range of test substances have been shown to influence angiogenesis in this model. Some of these affect tubule length and number, some tubule thickness, some the number of branch points and some a combination of these. Some of these are summarised in the table below

Selection of Molecules Shown to be Active in the TCS AngioKit Assay

Inhibitors	Enhancers
VEGF neutralising antibody	VEGF
VEGF antisense oligonucleotide	PIDGF
Collagen IV neutralising antibody	BFGF
PECAM-1 neutralising antibody	2-deoxyribose-1-P
VEGF-R antagonists	Most tumour cell conditioned media
Thrombospondin derivatives	Wound fluid
TNF alpha	Ascitic fluid from leiomyosarcoma patient
TGF β 1	Ascorbic acid (biphasic response)
α V β 3 integrin antagonists	
Suramin	
Curcumin	
Fumagillin	
TNP-740 (fumagillin analogue)	
Colchicine	
Cytochalasin B	
Erucic acid	
Ginsengosides	
RGD peptide	
Decorin	
Endostatin	
Thymine	
Thalidomide and analogues	
PAI-1 inhibitors	
MMP inhibitors	
MGBG (methylglyoxalbis(guanylhydrozone))	
Some conditioned media	

Note that this represents only the molecules whose identity we know. We have tested many proprietary compounds from various laboratories that were active in both senses but which were supplied as coded samples whose structure is not known to us.

Examples

Angiogenesis in the kit is greatly stimulated by addition of VEGF at 10ng/ml (Fig. 4) and inhibited by anti-VEGF neutralising antibody (not shown). Tubule development is strongly inhibited by Suramin. It is now known that Suramin's mode of action is to prevent VEGF binding to the KDR receptor (Kinase insert Domain containing Receptor) thus inhibiting tyrosine phosphorylation of KDR. Fig 5a shows that at doses of 200 μ M and 20 μ M, Suramin has a significant anti-angiogenic effect but at 2 μ M this is no longer seen.

TNF- α has also been shown previously to inhibit angiogenesis. When used in the AngioKit, TNF concentrations as low as 0.02 ng/ml showed a significant inhibitory effect (Fig 5b).

Presentation

The TCS angiogenesis model is presented as a kit comprising a pre-seeded 24 well plate containing all components necessary for tubule development and the medium required for medium changes during the period of tubule growth. The plate is shipped on day 2 or 3 of the co-culture and is completely filled with medium for transport. On receipt, the medium should be partially removed in accordance with the instructions enclosed and the cells allowed to equilibrate for 24 hours before further medium changes or reagent additions.

Accessory kits containing validated fixing and staining reagents are also available, as are positive and negative angiogenesis control reagents. Individual antibodies for IHC work can also be supplied separately (details on the following page).

A dedicated software package (AngioSys) for scoring image analysis of the assay is also available commercially. Please contact us for a free demo disc.

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TCS Angiogenesis Kit and Ancillary Reagents

Ordering Information

Code	Description	Quantity
ZHA-1000	Human Angiogenesis Model (AngioKit) 1x24 well plate plus all necessary medium changes	1 kit
ZHA-1100	AngioKit 'ELISA' Staining Kit (contains CD31 primary antibody, relevant secondary antibody and soluble substrate)	1 kit
ZHA-1125	Combined 'ELISA' & CD31 Tubule Staining Kit (ZHA-1100 & ZHA-1225)	1 kit
ZHA-1200	Angiogenesis Tubule Staining Kit (contains both CD31 and vWF antibodies, secondary antibodies and substrates)	1 kit
ZHA-1225	Angiogenesis Tubule Staining Kit (contains anti-CD31 primary antibody, secondary antibody and substrate)	1 kit
ZHA-1250	Angiogenesis Tubule Staining Kit (contains anti-vWF primary antibody, secondary antibody and substrate)	1 kit
ZHA-1300	Angiogenesis Control Reagent Kit (contains both Suramin and VEGF)	1 kit
<i>Additional materials</i>		
ZHA-1400	Additional optimised growth medium	100ml

NB: To run a complete assay with controls requires only ZHA-1000, ZHA-1300 and either ZHA-1225 or ZHA-1250

In House Assay Service

We are also able to run your test compounds in the assay in our laboratories. This service is run under GLP conditions and under conditions of complete confidentiality.

Data presented includes quantitative image analysis of tubules formed, statistical analysis of results, electronic images of tubules and a comprehensive final report. Please contact us to discuss your angiogenesis assay requirements.

Intellectual Property

Note that the AngioKit is protected by US patent 6225118, GB patent 2331763 and European patent 1023599. Other patents are pending

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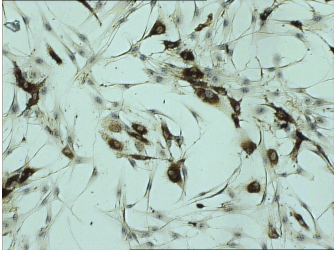


Fig 1a

Day 1 of tubule formation. Endothelial cells stained with antibody to von Willebrand factor (x100)

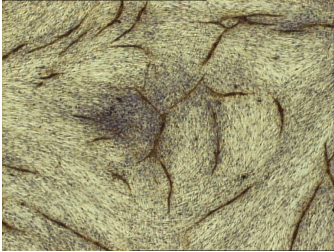


Fig 1b

Day 7 of tubule formation (x40)

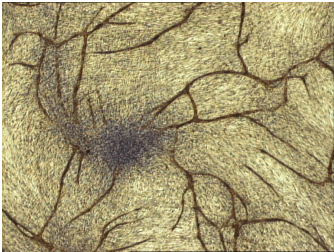


Fig 1c

Day 14 of tubule formation (x40)

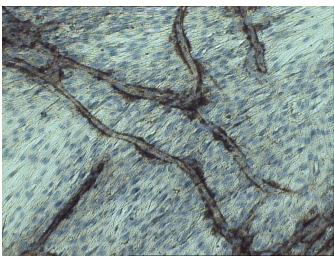


Fig 2

Tubules stained for collagen IV expression



Fig 3

Tubules stained for PECAM-1 expression

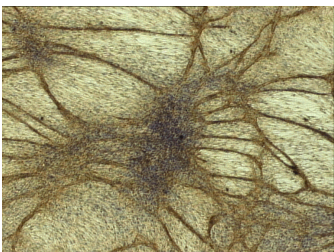


Fig 4

Stimulatory effect of VEGF (10ng/ml). Stained for von Willebrand Factor expression

