

# *TCS CellWorks Ltd*

***TCS CellWorks Ltd***

*the first in vitro*

**“Human Angiogenesis Assay Kit”**

# Angiogenesis: the development of new capillaries from existing vasculature

Angiogenesis has important physiological roles in:

- Embryogenesis
- Female reproductive cycle
- Bone formation
- Wound healing

However, angiogenesis is quiescent in the normal adult and its inhibition is considered unlikely to cause adverse effects

# *Pathological aspects of angiogenesis:*

## Excessive Angiogenesis:

- Tumour development and progression
- Diabetic retinopathy
- Rheumatoid arthritis
- Psoriasis

## Diminished Angiogenesis:

- Wound healing
- “Biological bypass”

# Angiogenesis is a multi-step process:

- Endothelial cell activation
- Proliferation of endothelial cells
- Migration of endothelial cells
- Penetration of extracellular matrix
- Reorganisation of cells into tubules (or columns)
- Formation of a lumen (apoptosis?)
- Anastomosis

# Existing *in vivo* models/assays for angiogenesis:

- Chick chorioallantoic membrane assay (CAM)
- Rodent cornea assay
- Subcutaneous air sac in rodents
- Sponge and gel implants
- Rat freeze-injured skin graft assay

# Existing in vitro 'angiogenesis' assays

- Endothelial cell proliferation assays
- Endothelial cell migration assays
- Tubule formation assays (usually in Matrigel or similar)
- Matrix metalloproteinase release assay

# *TCS CellWorks Ltd*

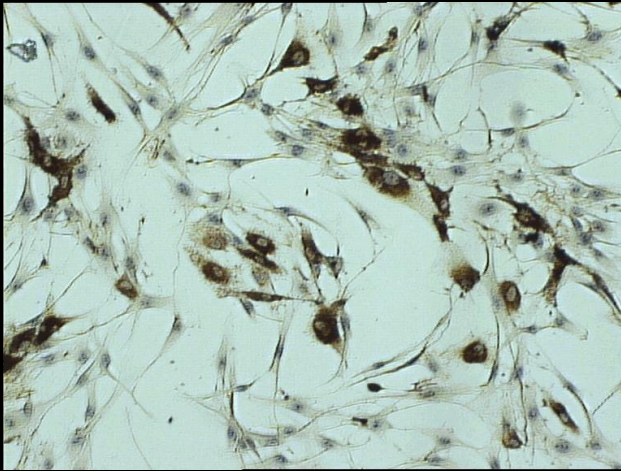
## **Aim:**

To develop an *in vitro* all human model of angiogenesis that includes all the known phases of the angiogenic process

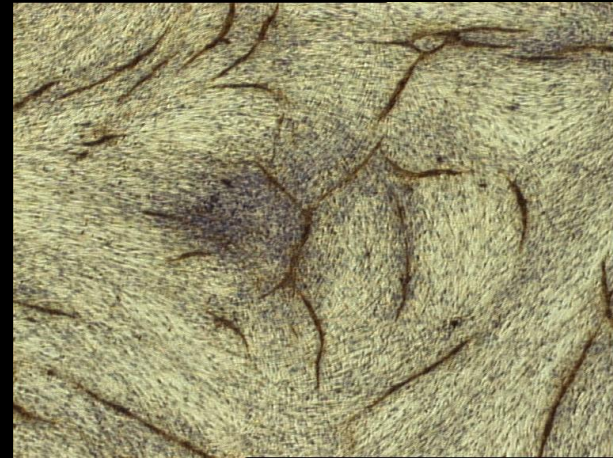
# TCS human angiogenesis assay:

- Co-culture of primary human endothelial cells with primary human interstitial cells
- No other additions or manipulations except addition of test substances and medium changes
- Anastomosing tubule network forms in 10-14 days
- Tubules stained with antibodies to von Willebrand factor, PECAM-1 or collagen type IV
- Permits evaluation of both inhibitors and stimulators of angiogenesis
- 24 well format - compatible with replicate samples or secondary screening needs
- Several approaches to analysis of results are available (tubule length, branch points, tubule area etc)

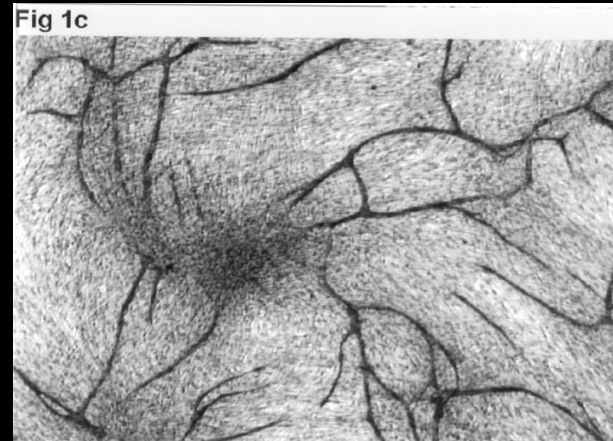
# *TCS Angiogenesis Assay*



*Day 1*



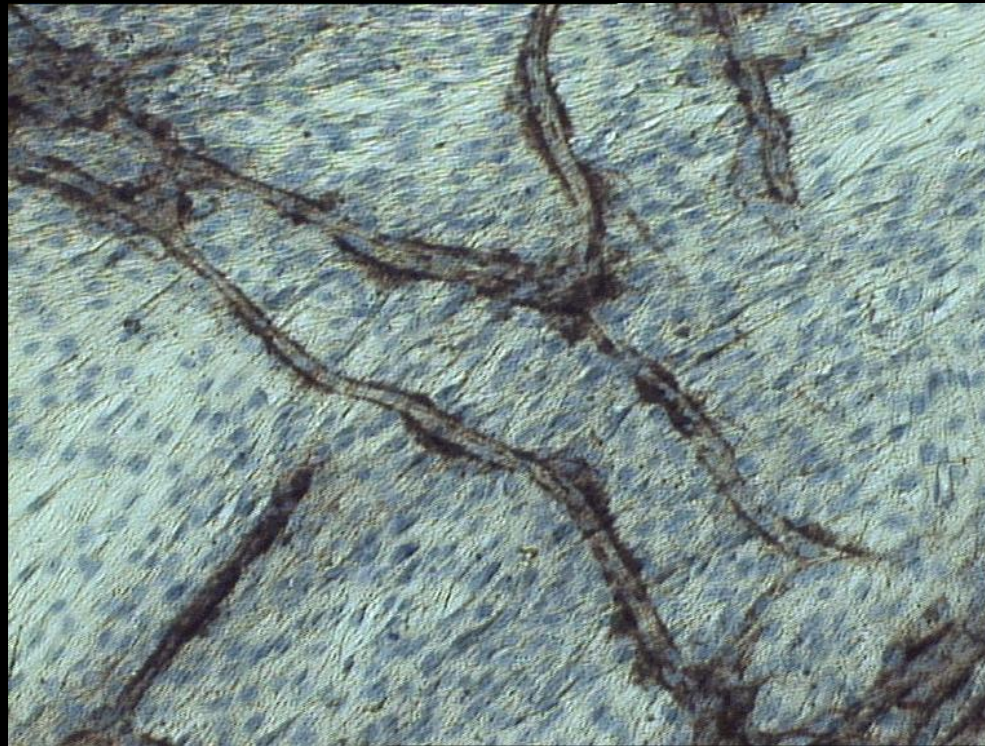
*Day 7*



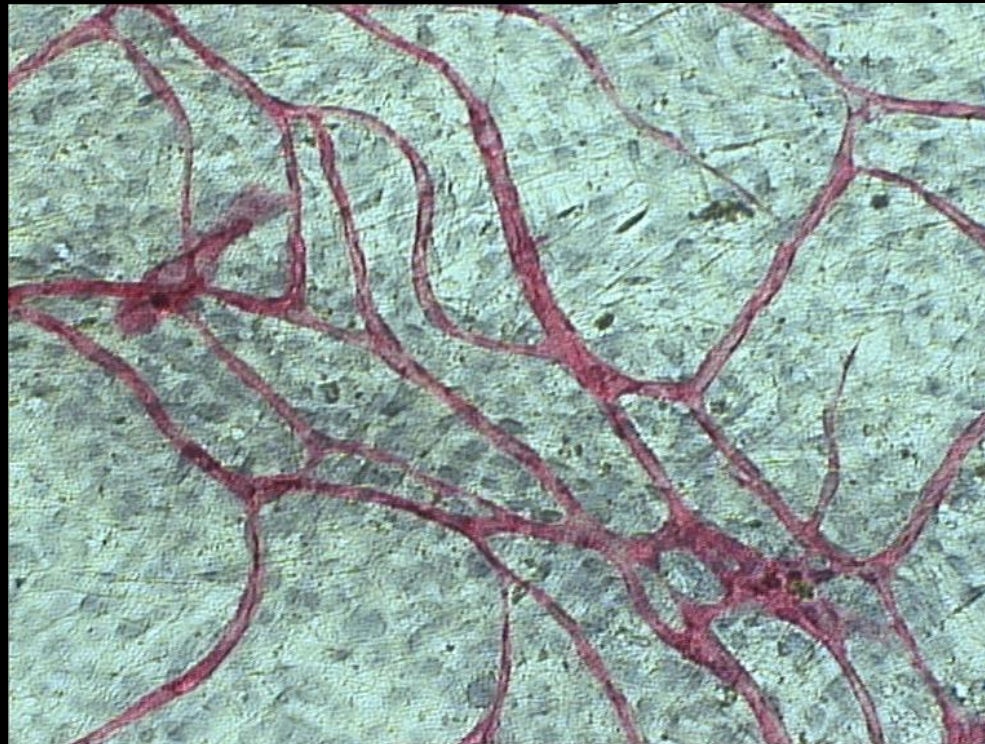
*Day 14*

*Stained: anti-von Willebrand Factor*

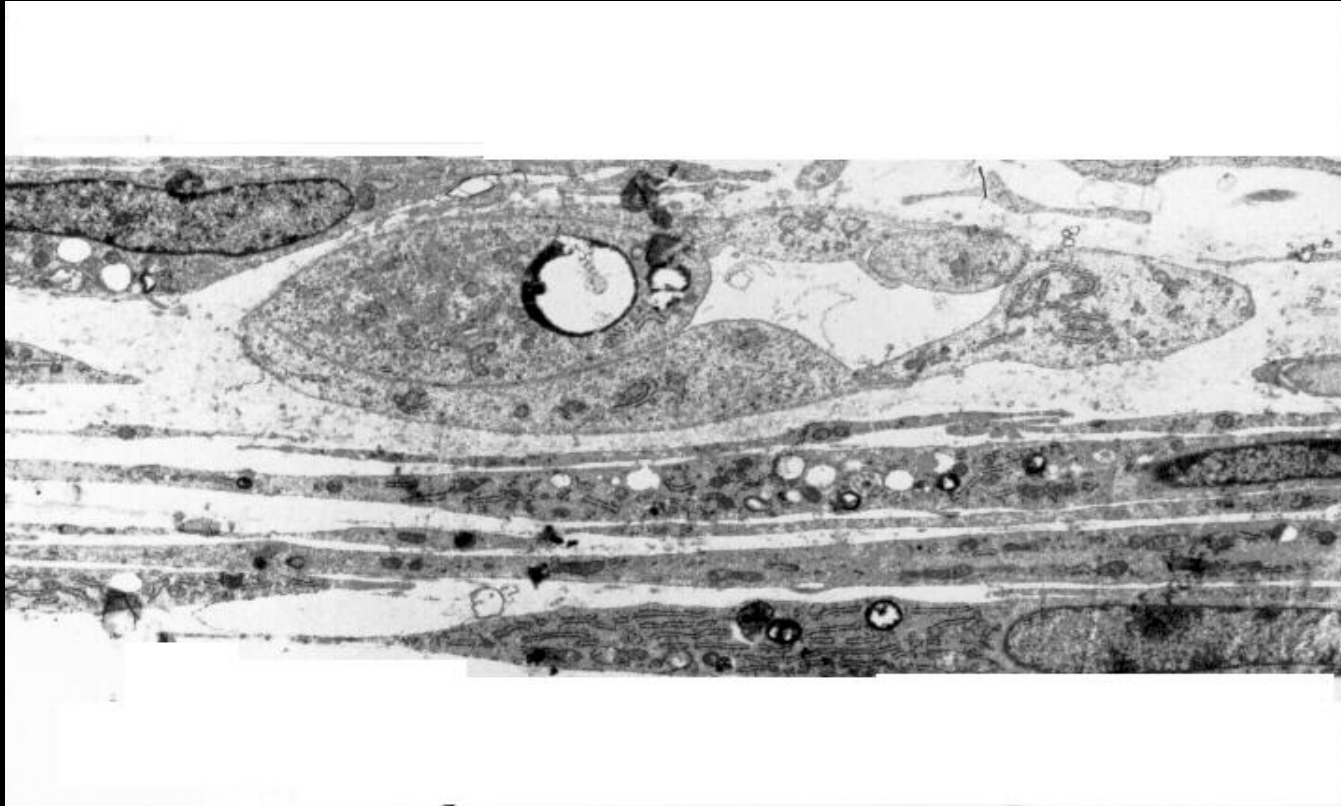
# Tubules stained with anti-collagen type IV



# Tubules stained with anti-PECAM-1

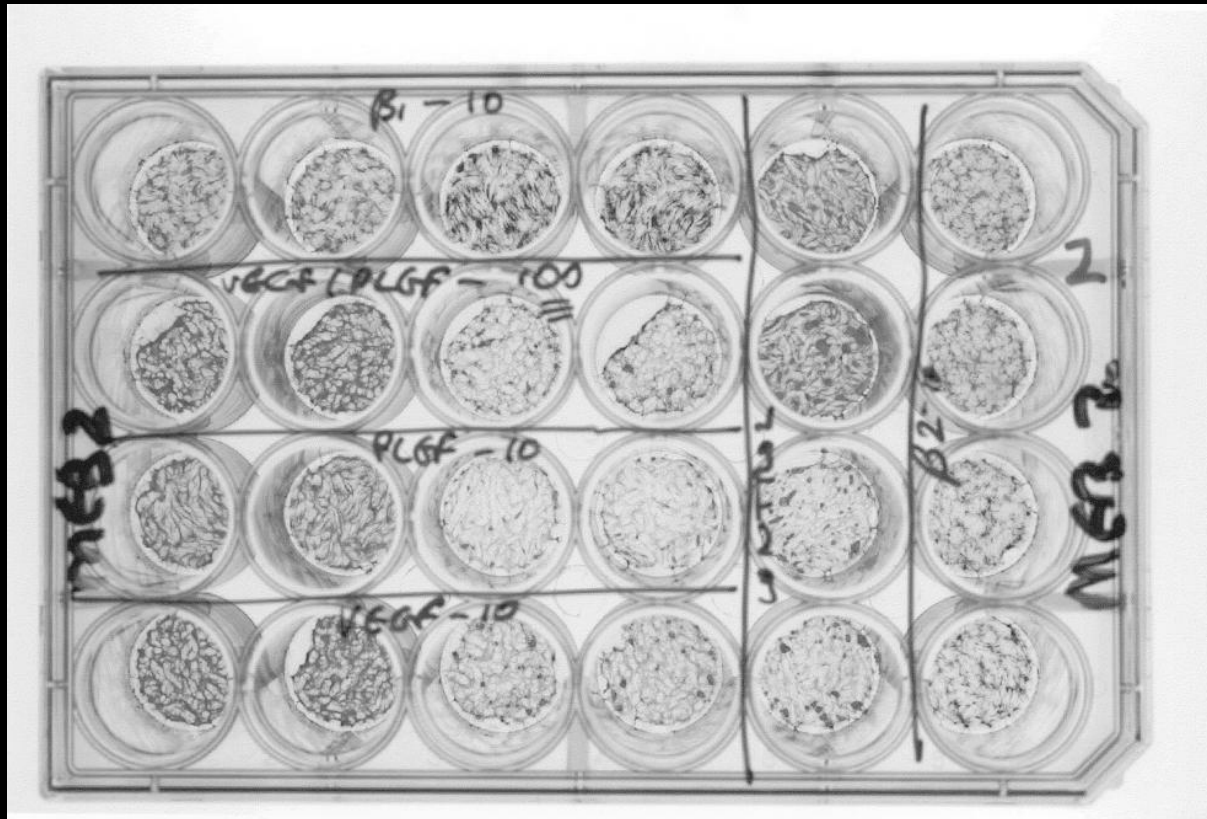


# Tubule Cross-section



# *TCS Angiogenesis Assay*

*Whole plate view*



# *TCS Angiogenesis Assay*

Suramin 20 $\mu$ M



Control



VEGF 2ng/ml



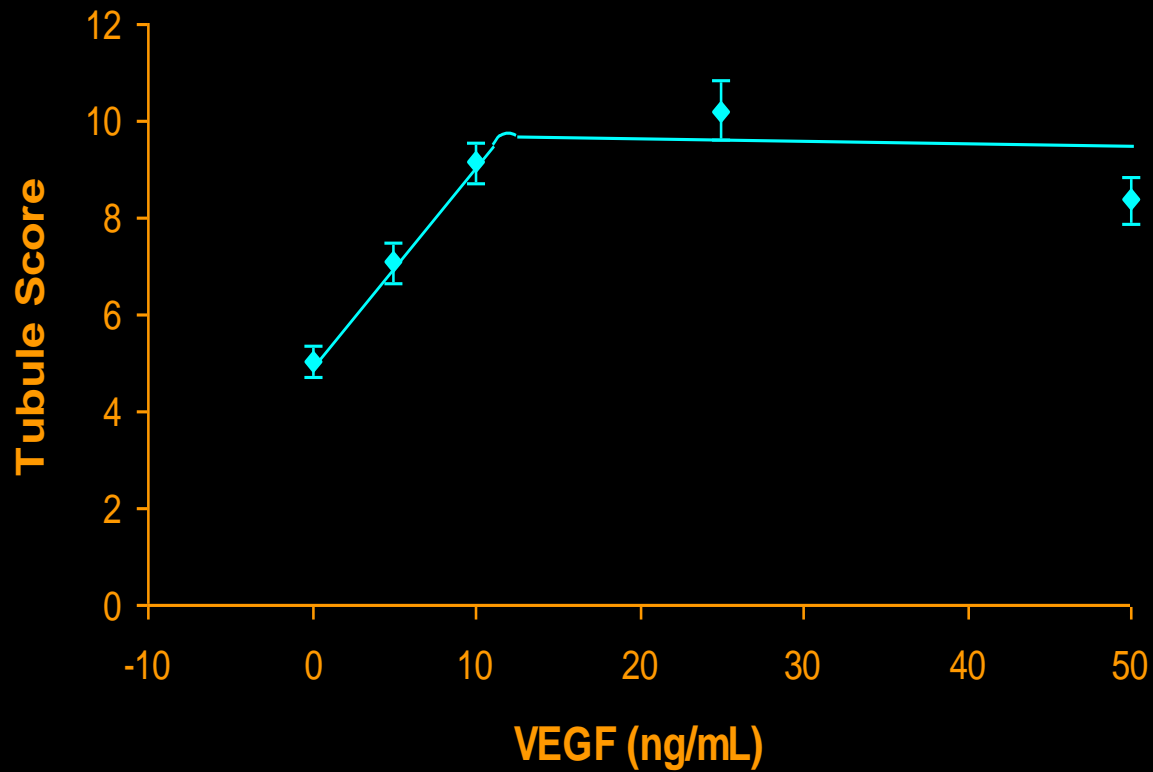
# *Angiogenesis Assay Quantitation*

- Image analysis
  - tubule length
  - branch points
  - (area)
- Chalkley graticule
- Map reader
- Optical density

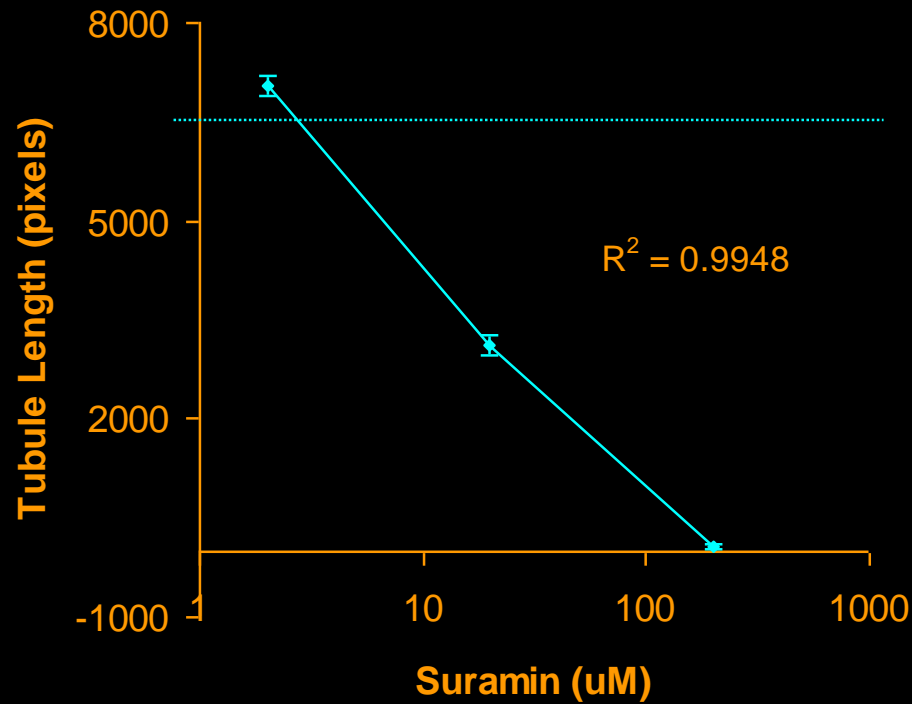
# *Angiogenesis Assay Options*

- Buy kits for in house use
- Have tests performed by TCS
- Combination - initial tests at TCS then bring in house when usage warrants it

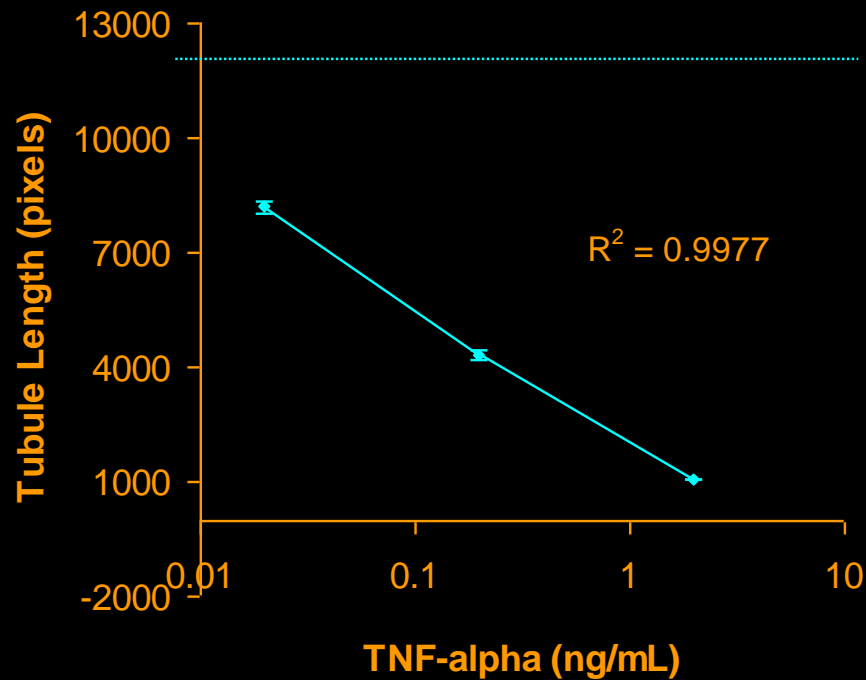
# *Tubule stimulation by VEGF*



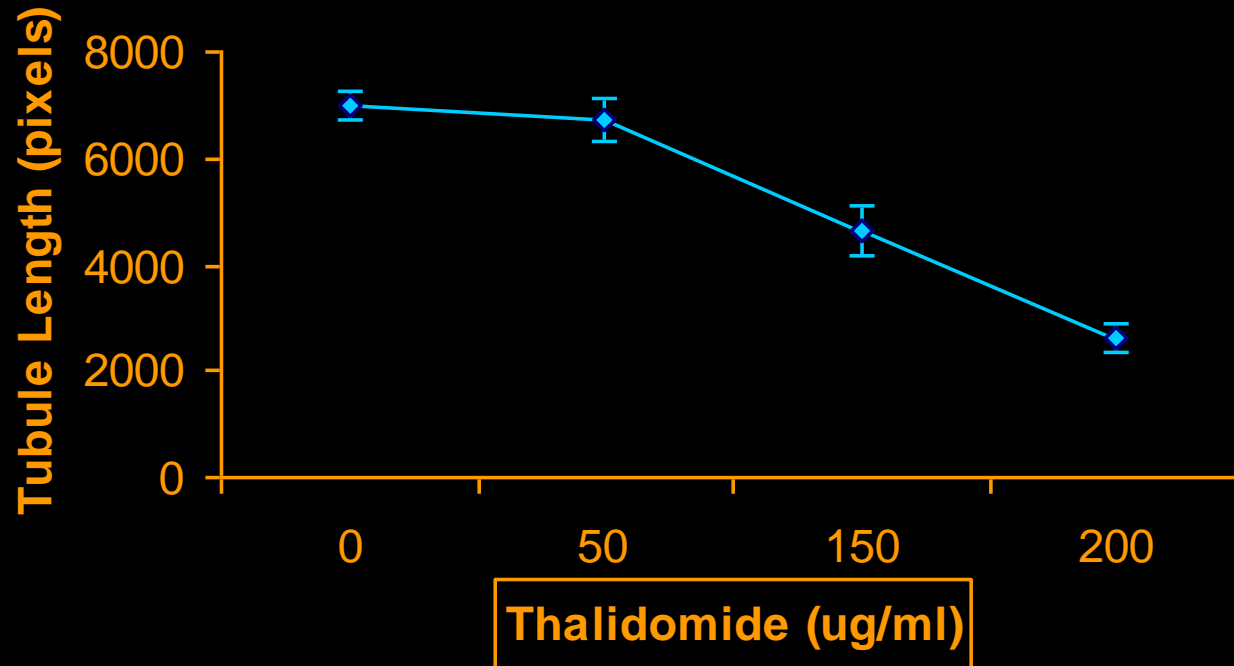
# *Effect of suramin concentration on tubule formation*



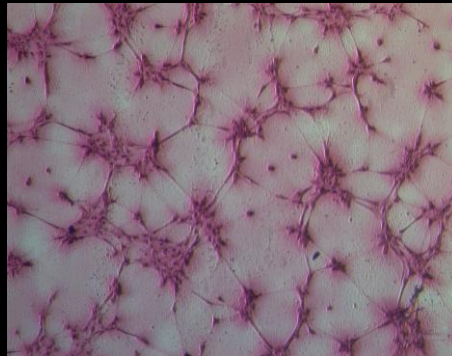
## *Effect of TNF alpha on tubule formation*



## Effect of Thalidomide on Angiogenesis



# *Artefactual tubule formation in Matrigel*

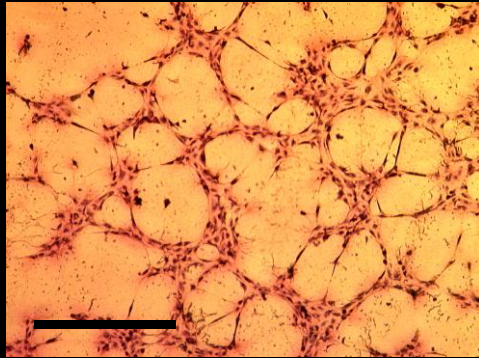
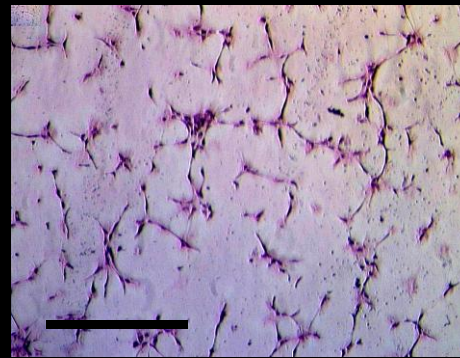
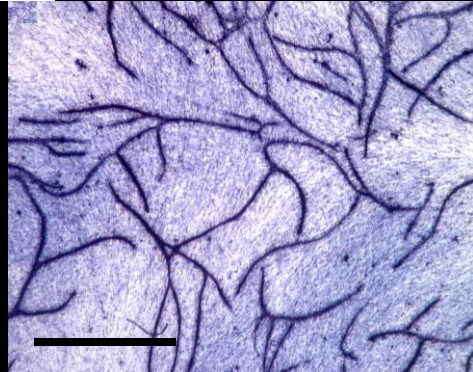
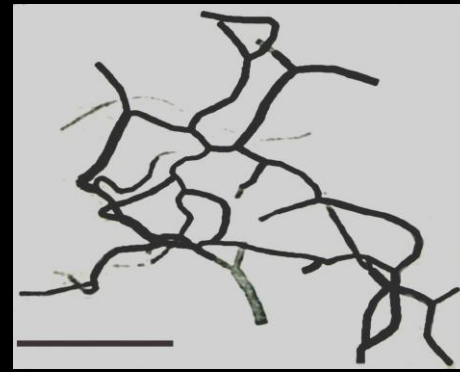


**A**



**B**

Tubule-like structures formed by fibroblasts (**A**) and MDA-MB-231 breast cancer cell line (**B**) on GFR Matrigel

**A****B****C****D**

Tubules formed on standard Matrigel (A), GFR Matrigel (B) and in the TCS CellWorks angiogenesis kit (C) compared to capillaries in 3-D reconstruction of a microvascular network *in vivo* (D). Scale bar = 100  $\mu$ m.

# Some agents active in the TCS angiogenesis assay

## Inhibitors

Antibody to VEGF  
VEGF antisense oligonucleotide  
Integrin  $\alpha$ V  $\beta$ 3 antagonists  
Suramin  
Curcumin  
Anti-VEGF-R  
TGF $\beta$ -1  
2-deoxy-ribose  
Matrixmetalloproteinase inhibitor

Thalidomide analogues

## Enhancers

VEGF  
bFGF  
Placenta Derived Growth Factor  
Thymine  
Tumour cell conditioned medium  
(hypoxia dependant)  
Wound fluid

## *In conclusion*

- An assay that can quantify both positive and negative modulators of angiogenesis
- An assay that reflects all phases of the angiogenic cascade
- An assay adapted to multiple samples
- An all human assay