The role of oxygen insufficiency in the onset and development of the vascular complications of diabetes

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Where?

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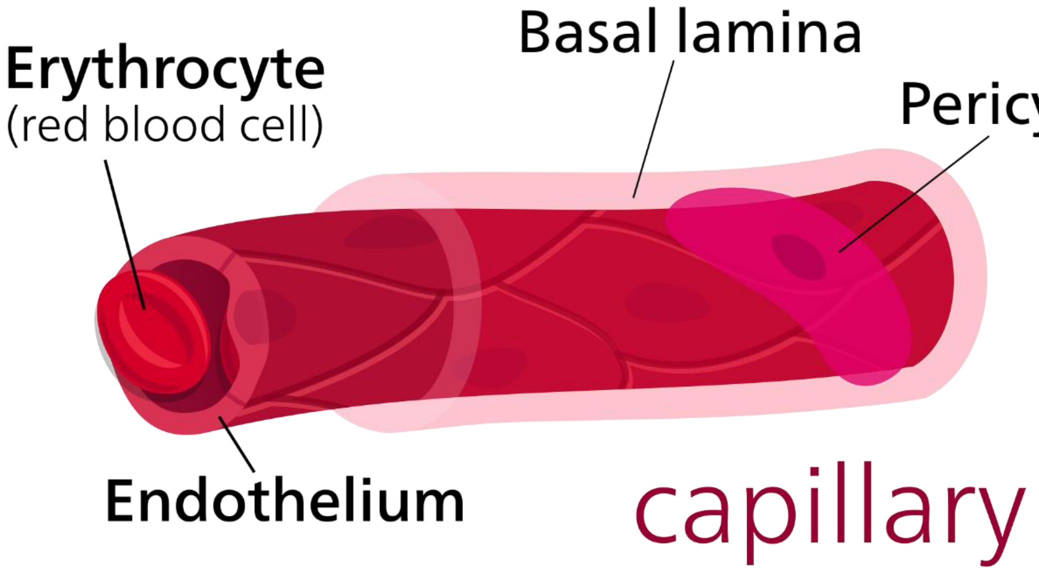
Oxygen and cell survival

– Capillaries bring oxygen and nutrients to cells



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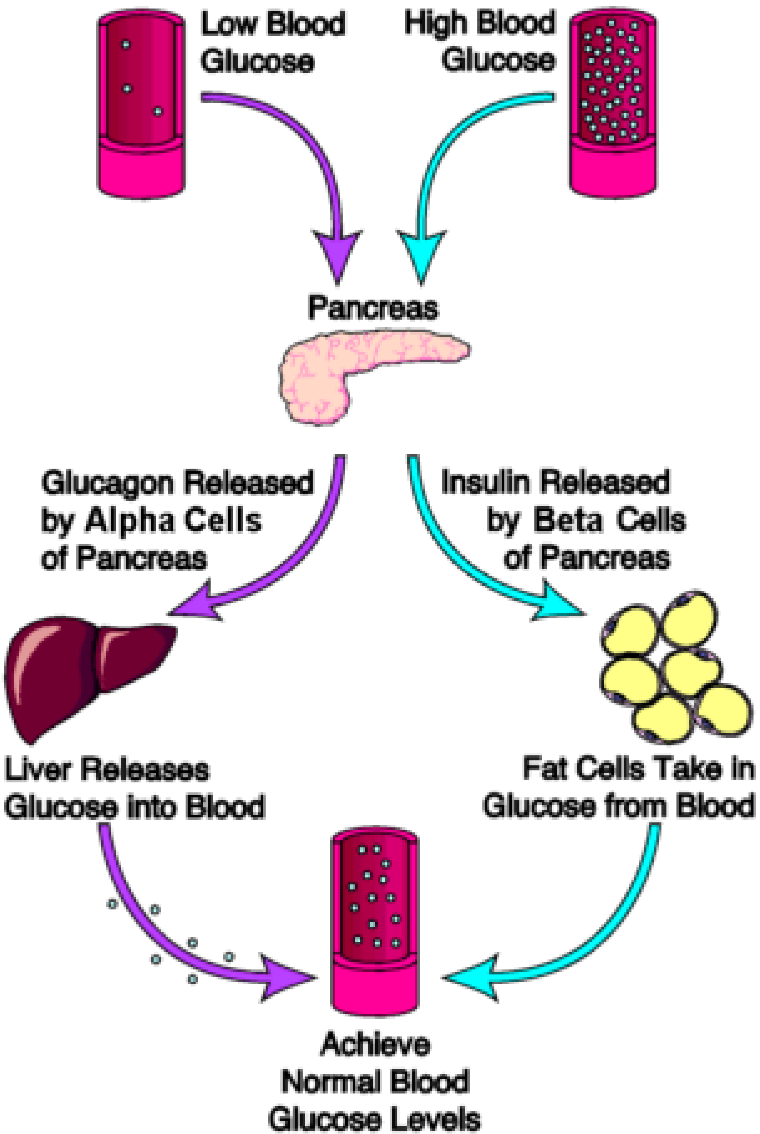
Glucose concentration



– Glucose concentration regulated by insulin

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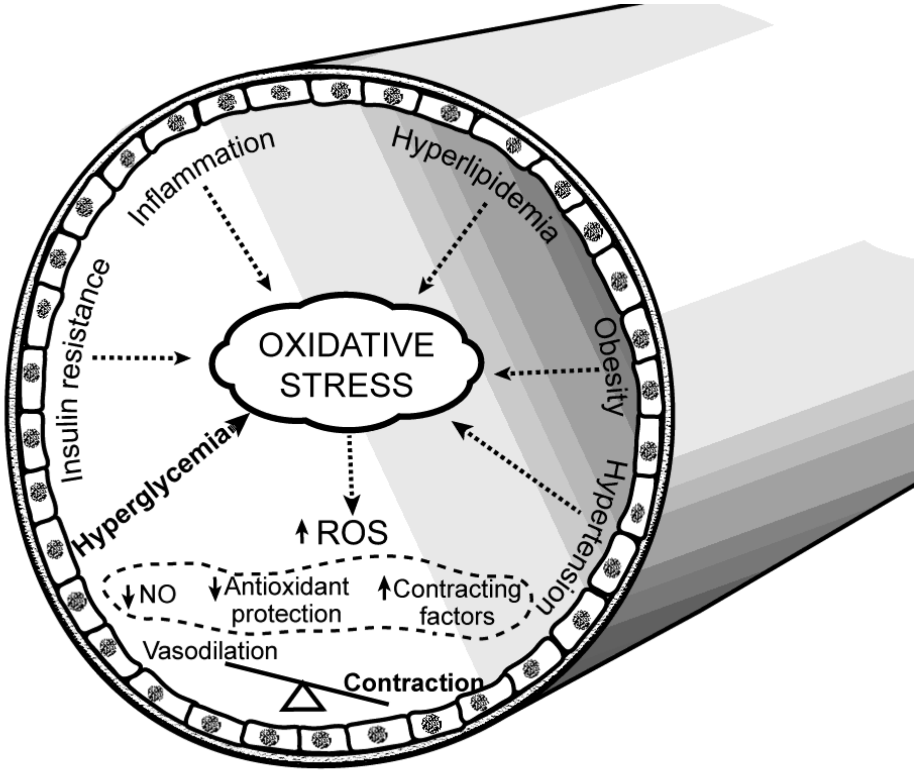
Unregulated glucose level

- Diabetes mellitus (DM)
  - Type 1 DM – insulin insufficiency
  - Type 2 DM – insulin resistance

- Clinical management of glucose levels is a priority

- High and/or fluctuating high levels of glucose increase the vascular complications resulting from the disease.

Glucose mediated endothelial cell damage



- Oxidative stress
- ↓ cell proliferation
- ↑ activation/adhesion
- Impaired O2 delivery
- Anaerobic glucose metabolism

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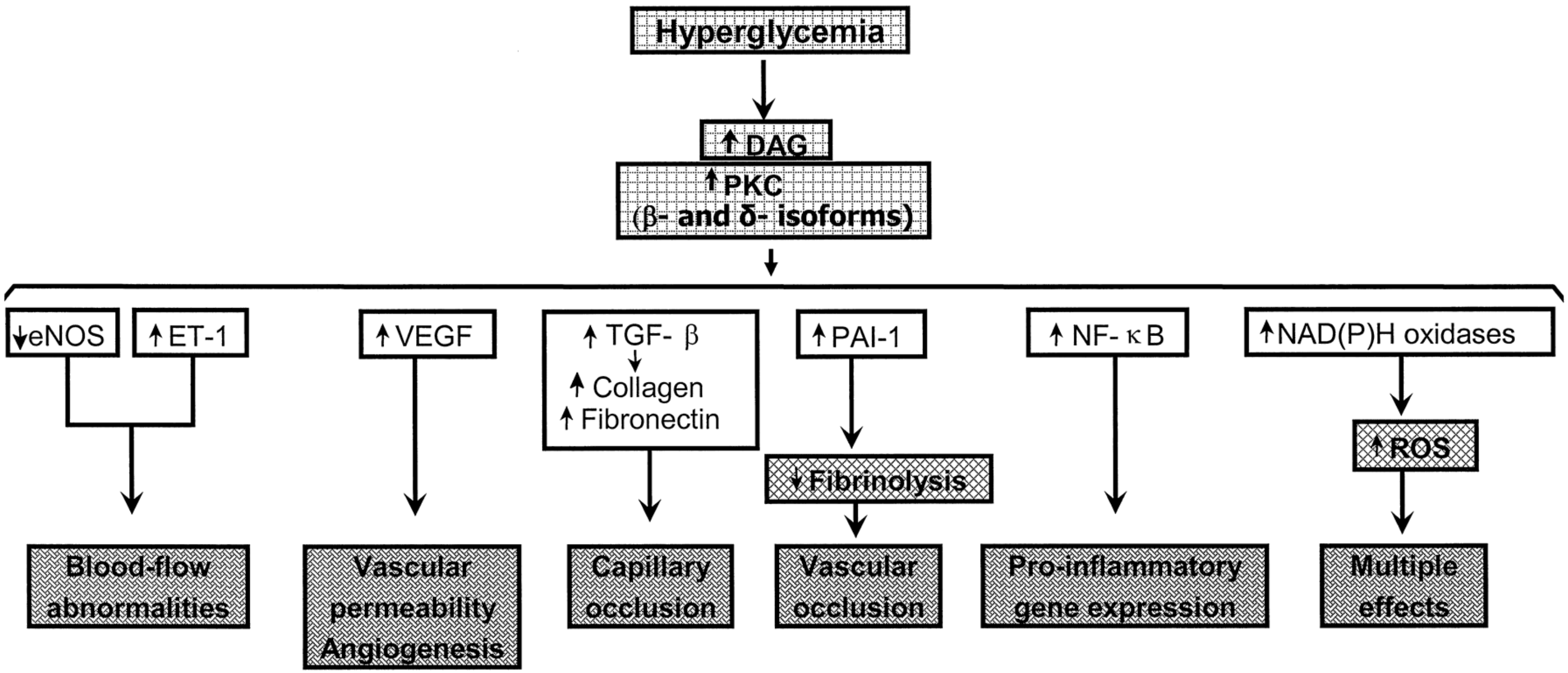
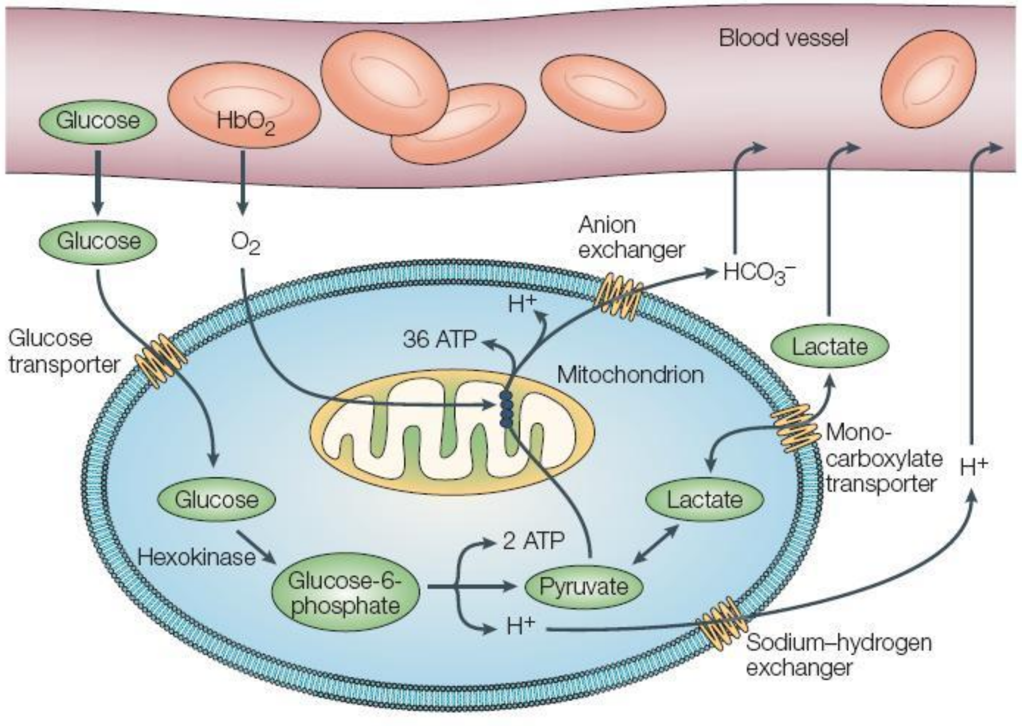


Hyperglycemia
↑DAG
↑PKC
(β- and δ- isoforms)
↓eNOS
↑ET-1
↑VEGF
↑TGF- β
↑Collagen
↑Fibronectin
↑PAI-1
↓Fibrinolysis
↑NF- κB
↑NAD(P)H oxidases
↑ROS
Blood-flow abnormalities
Vascular permeability Angiogenesis
Capillary occlusion
Vascular occlusion
Pro-inflammatory gene expression
Multiple effects

Anaerobic vs aerobic metabolism



Robert A. Gatenby & Robert J. Gillies
Nature Reviews Cancer 4, 891-899 (2004)

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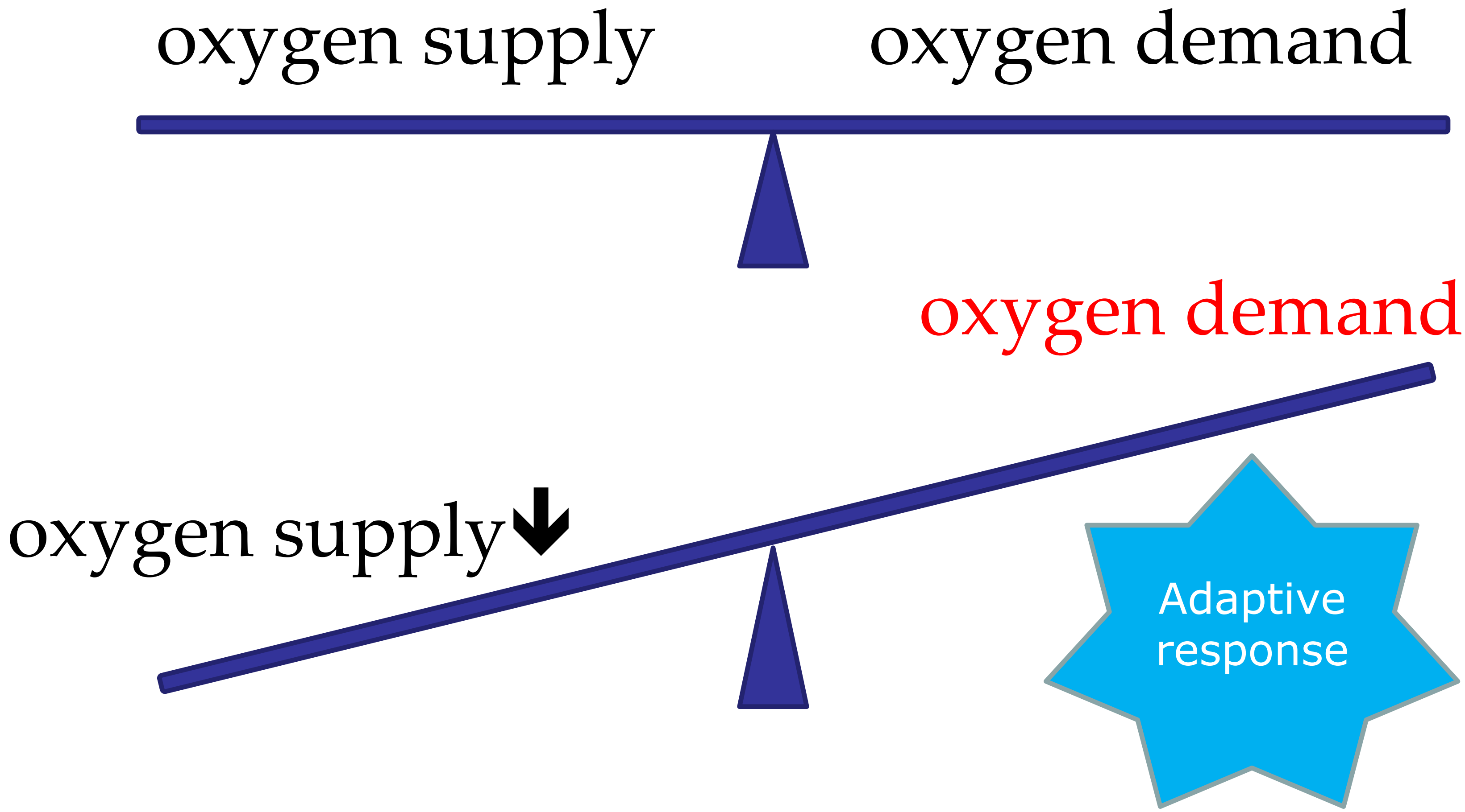
Oxygen insufficiency



Adaptive response to low oxygen

Consequence of normoglycaemia and low oxygen?	Rationale for the adaptive response	Consequence in hyperglycaemia and low oxygen?
> anaerobic metabolism therefore reduced ATP production	to increased glucose uptake to redress balance and increase ATP production	glucose levels may be already very high and increase oxidative stress
> glucose metabolism	to increased glucose metabolism to redress balance and increase ATP production	
> endothelial cell proliferation	to increase vascular areas and thus enhance oxygen delivery	hyperglycaemia modifies some of the structures within cells that are able to respond to mediate cell proliferation reducing ability of cells to proliferate
> erythropoietin production	to increase oxygen delivery to the cells/tissue	

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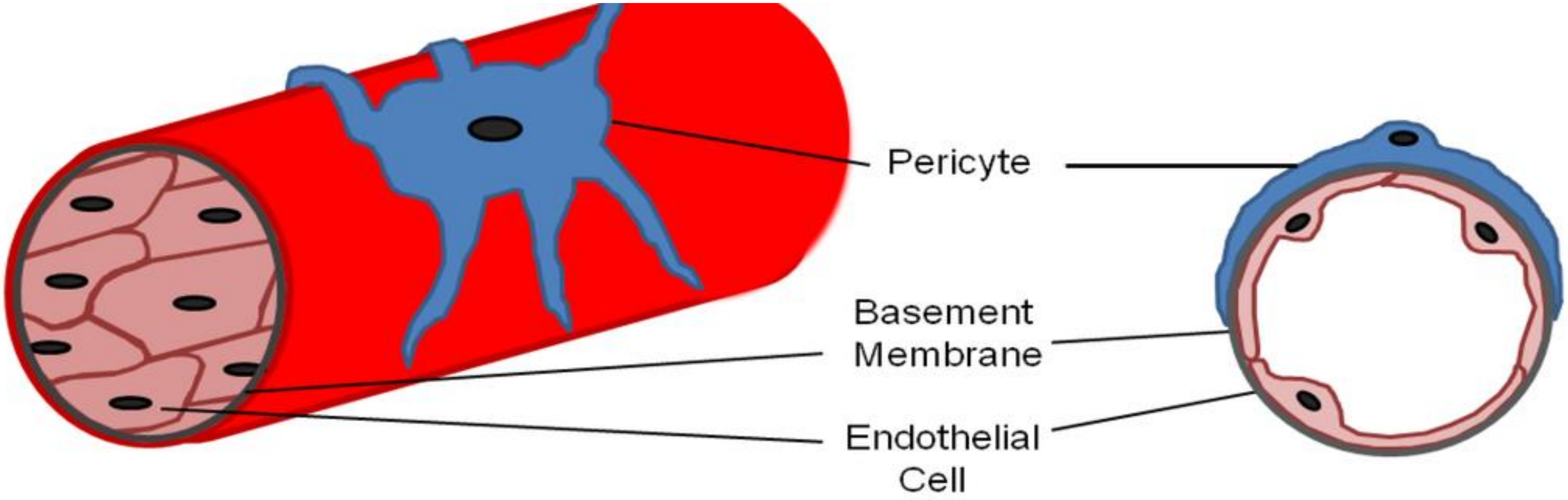
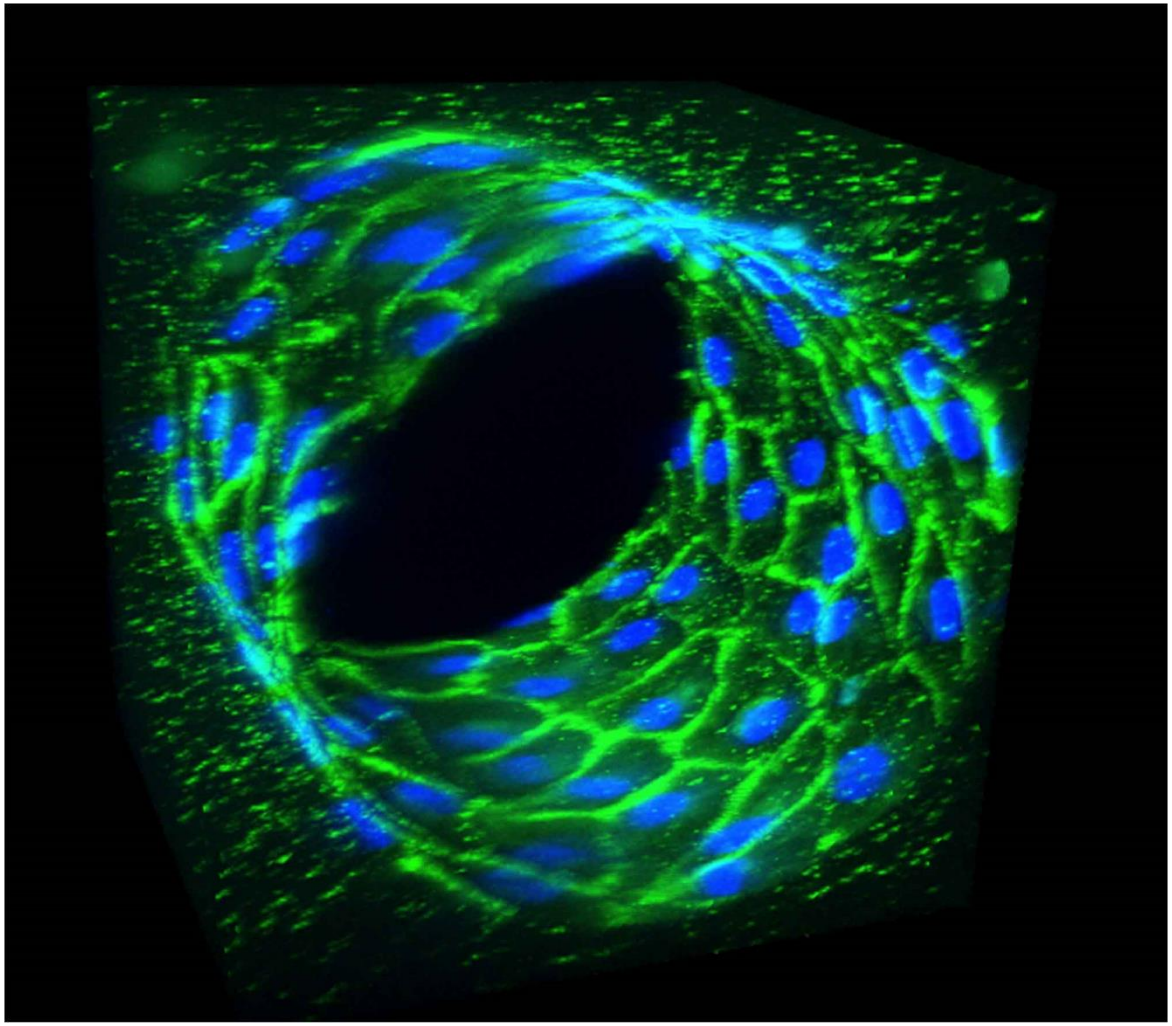
Capillary network



Endothelial cells



3D projection of a confocal z-stack shows human umbilical vein endothelial cells (HUVECs) forming a functional vessel immunofluorescently stained for PECAM-1 (green) and nuclei (blue). (Wong/Searson Lab)

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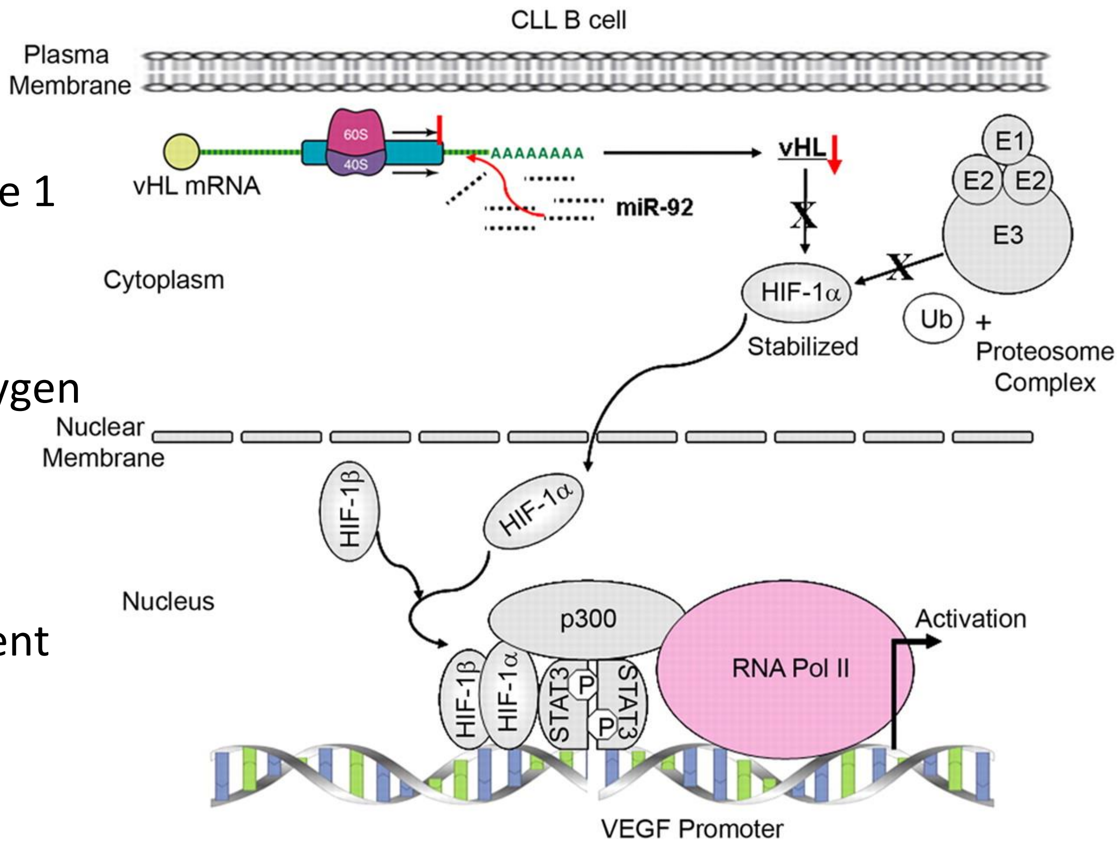
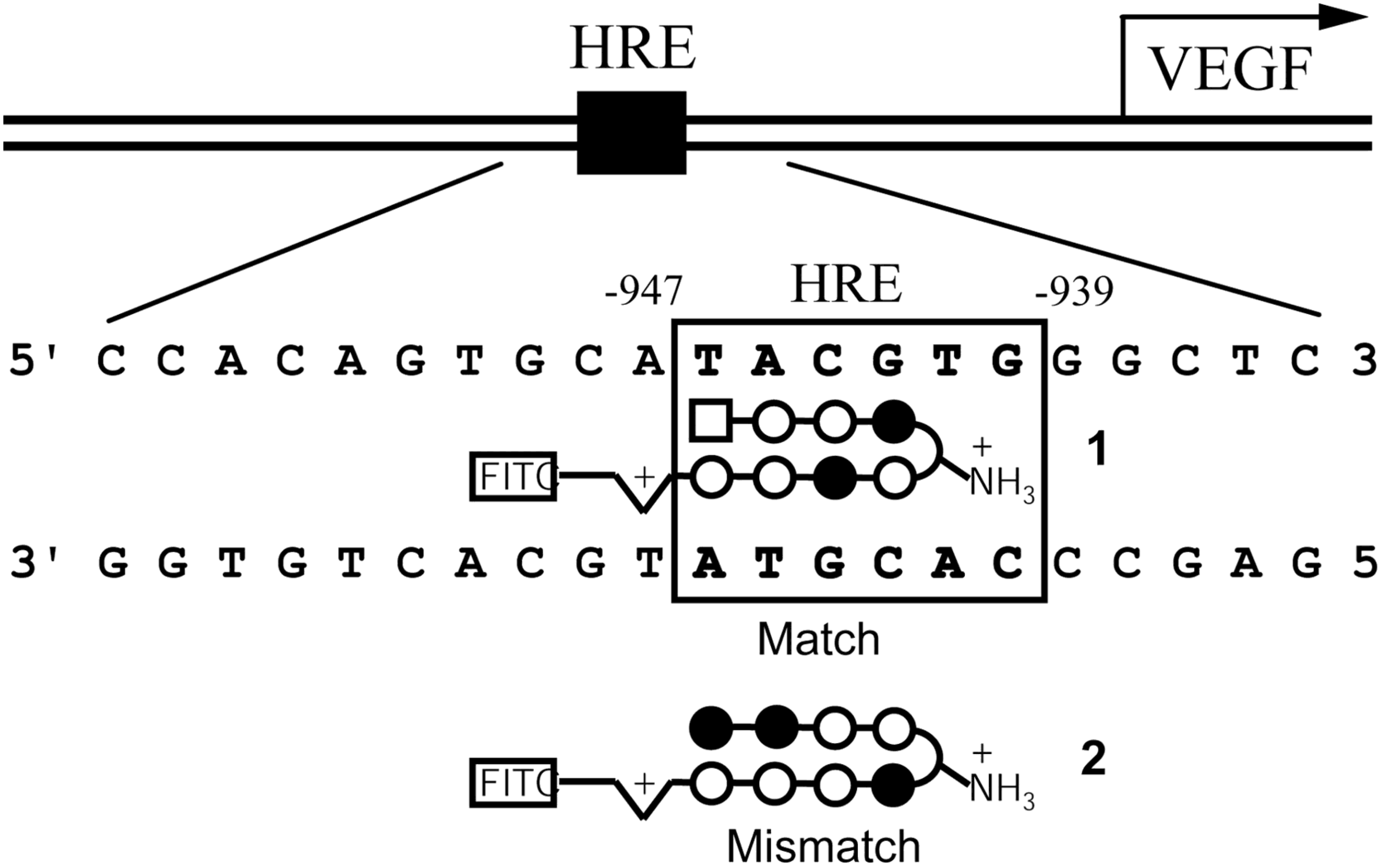
Capillary damage/dysfunction

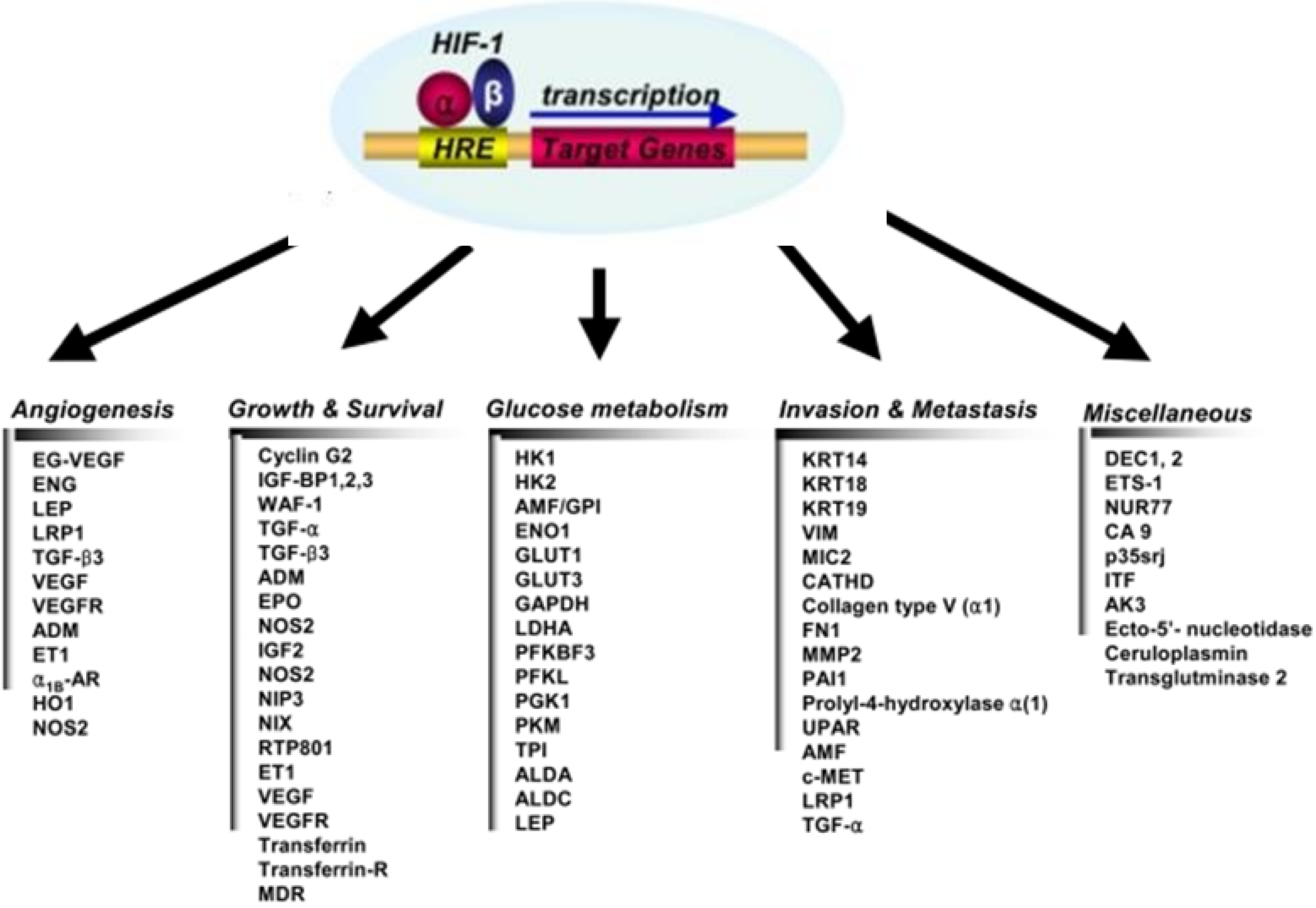
- Oxygen insufficiency
  - Hypoxia inducible factor type 1 (HIF1)
  - Dimeric transcription factor
    - HIF1α – stabilised in oxygen insufficiency
    - HIF1β – constitutively expressed
- Binds to hypoxia response element (HRE)



Hypoxia response element



Hypoxia inducible factor type 1



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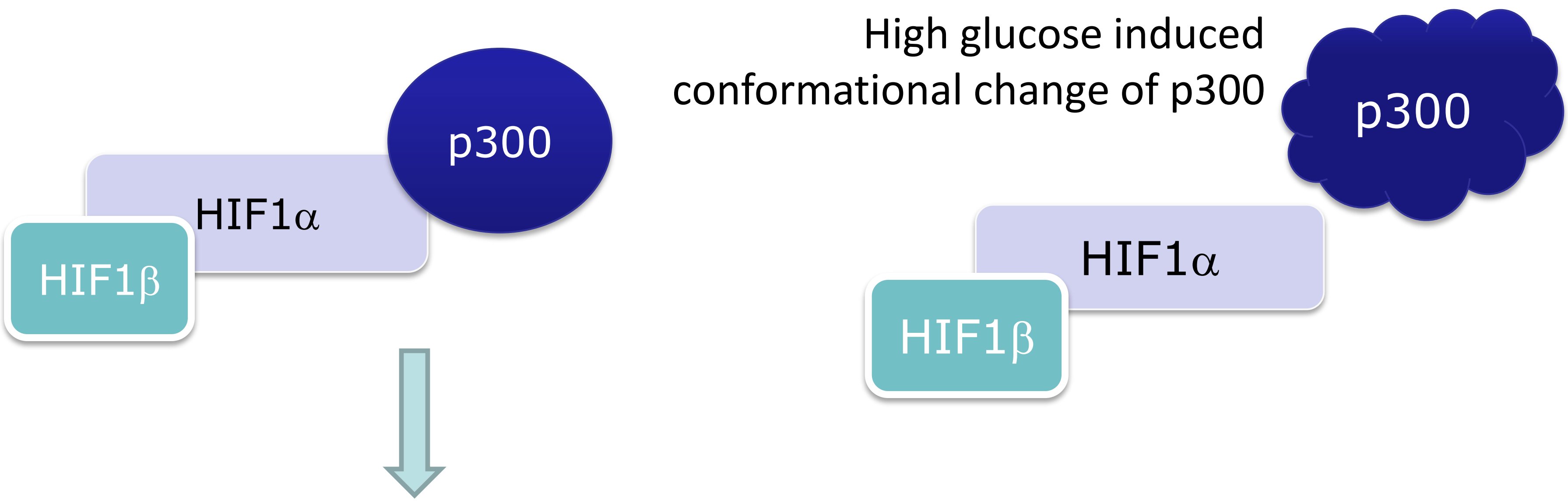
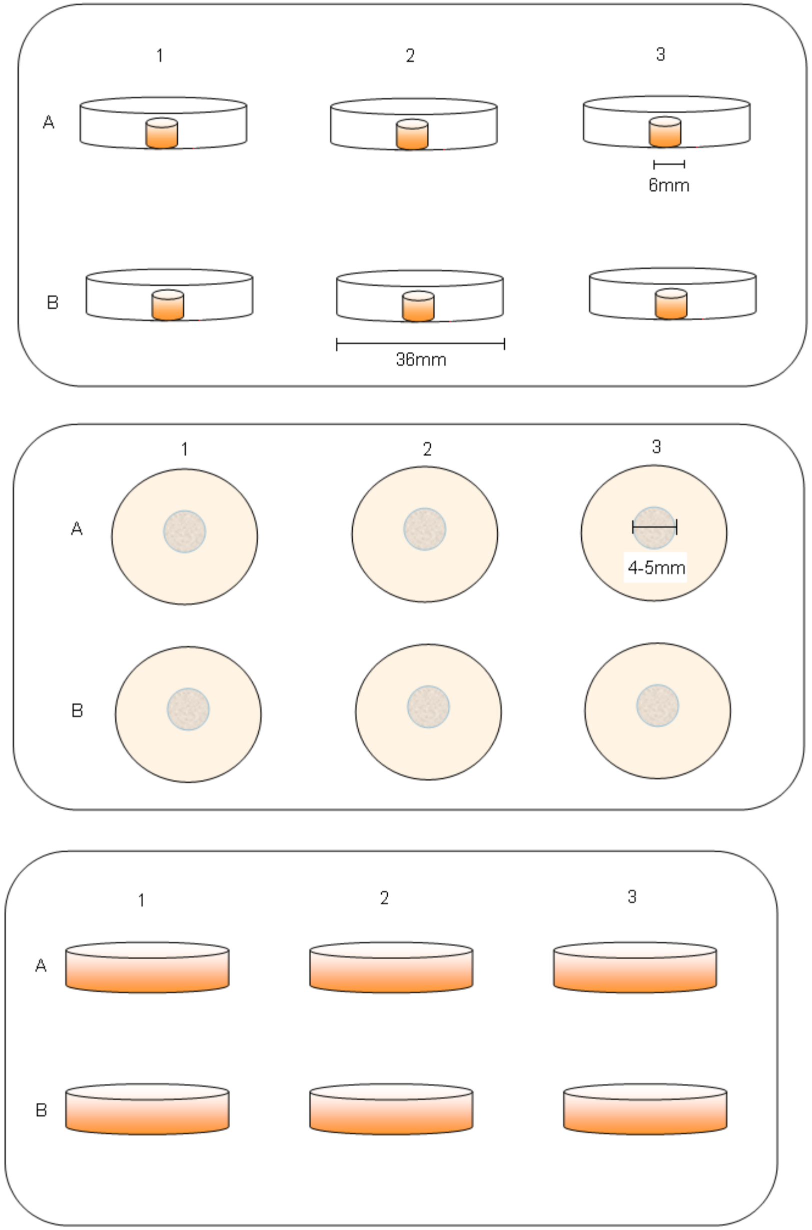
Glucose mediated change to HIF1 function

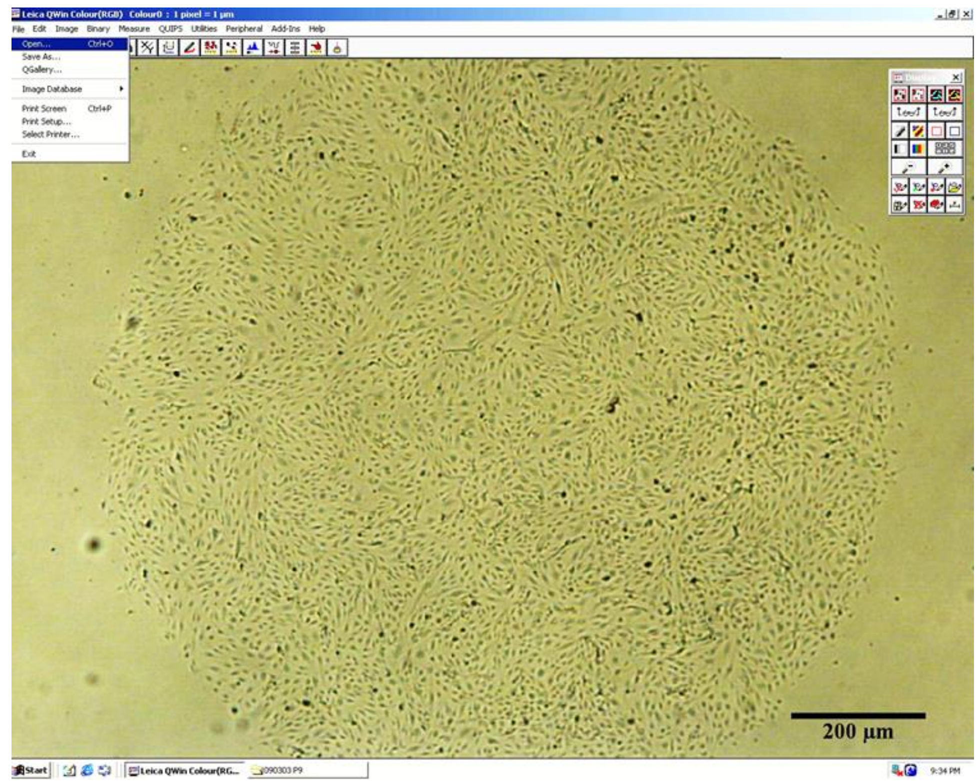


Activation of transcriptional activity

HIF-1α dysfunction in diabetes Cell Cycle 9:1, 75-79; January 1, 2010; Hariharan Thangarajah, Ivan N. Vial, et al.

Human microvascular dermal endothelial cell model





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Measurement & analysis

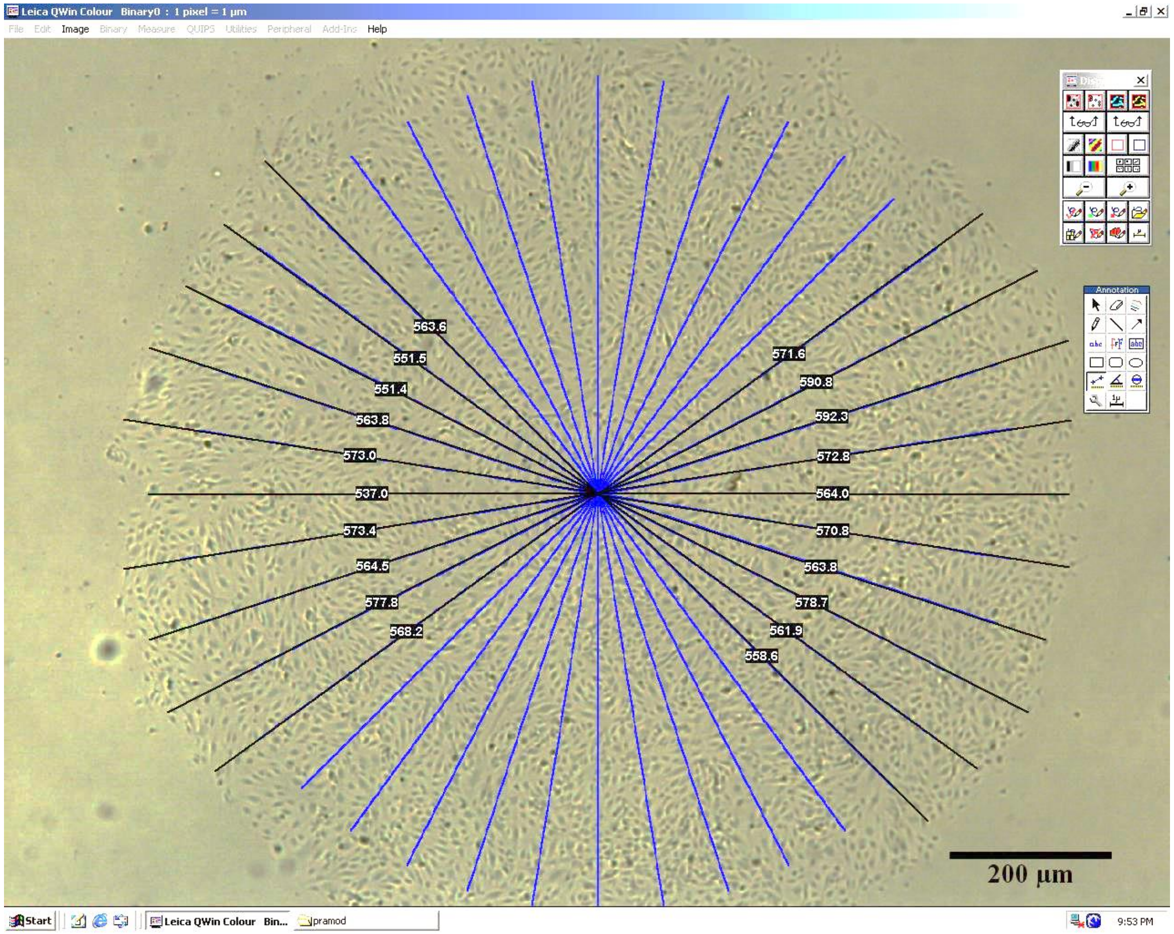

Image taken each day and stored for subsequent analysis

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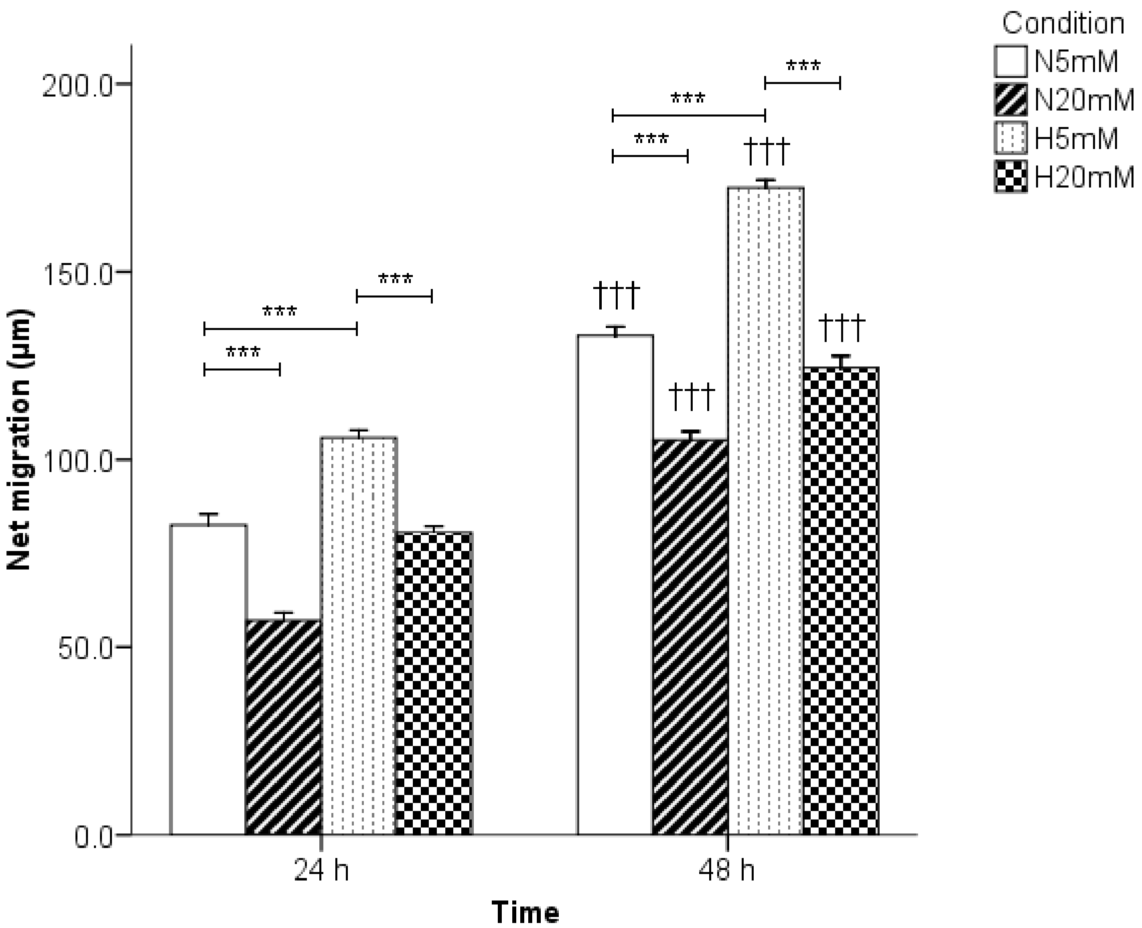
Effect of [glucose] and [oxygen]



Summary

Hypoxia increased net migration distance at 24 & 48 h.

High glucose concentration decreased net migration of endothelial cells at 24 & 48 hours.

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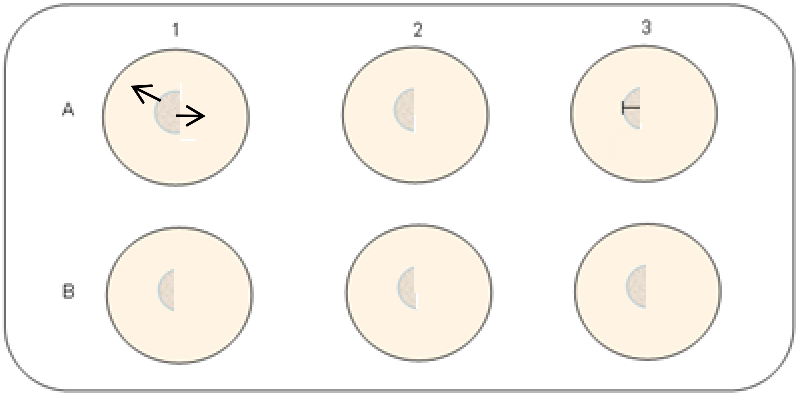
Gadad, Matthews & Knott

Human microvascular dermal endothelial cell (HMVDEC) model

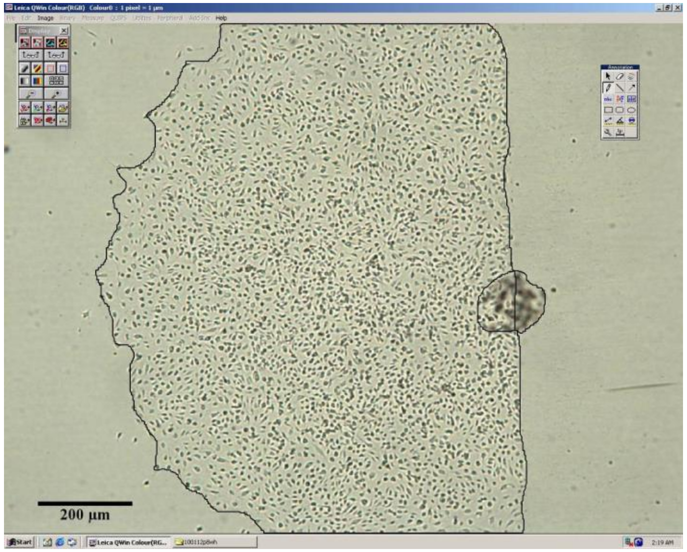
Normoxia
(18% oxygen)

Hypoxia
(5% oxygen)



5mM glucose

20mM glucose



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Measurement & analysis

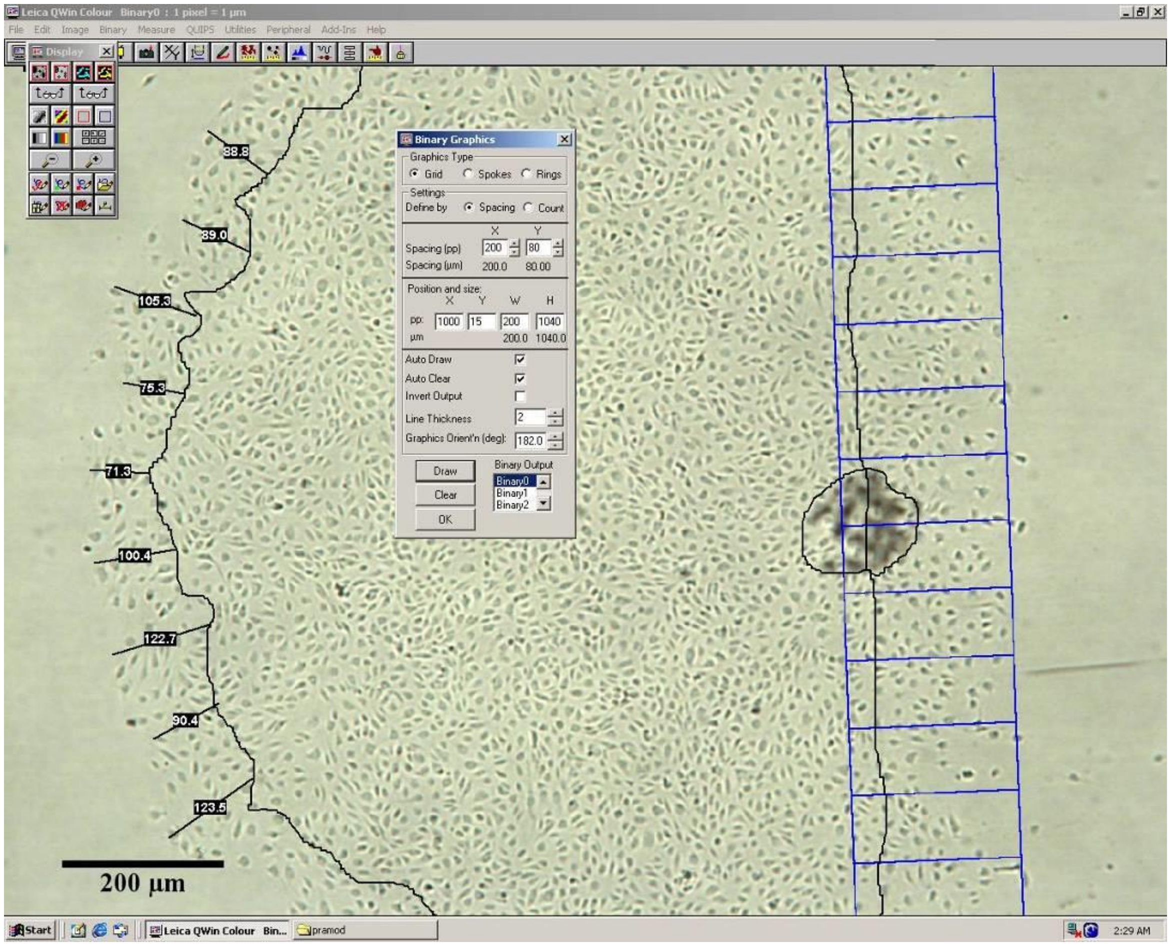

Image taken each day and stored for subsequent analysis

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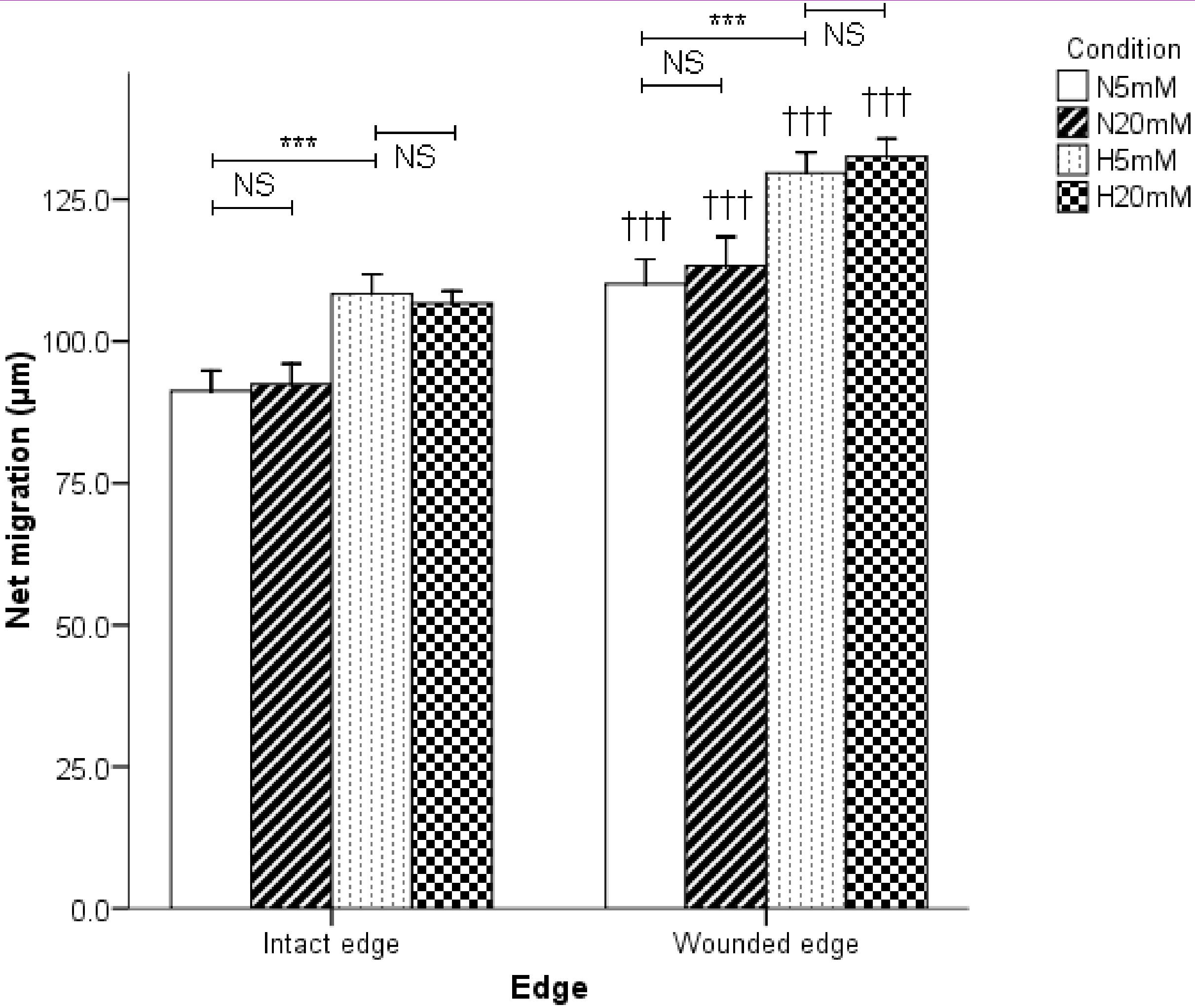
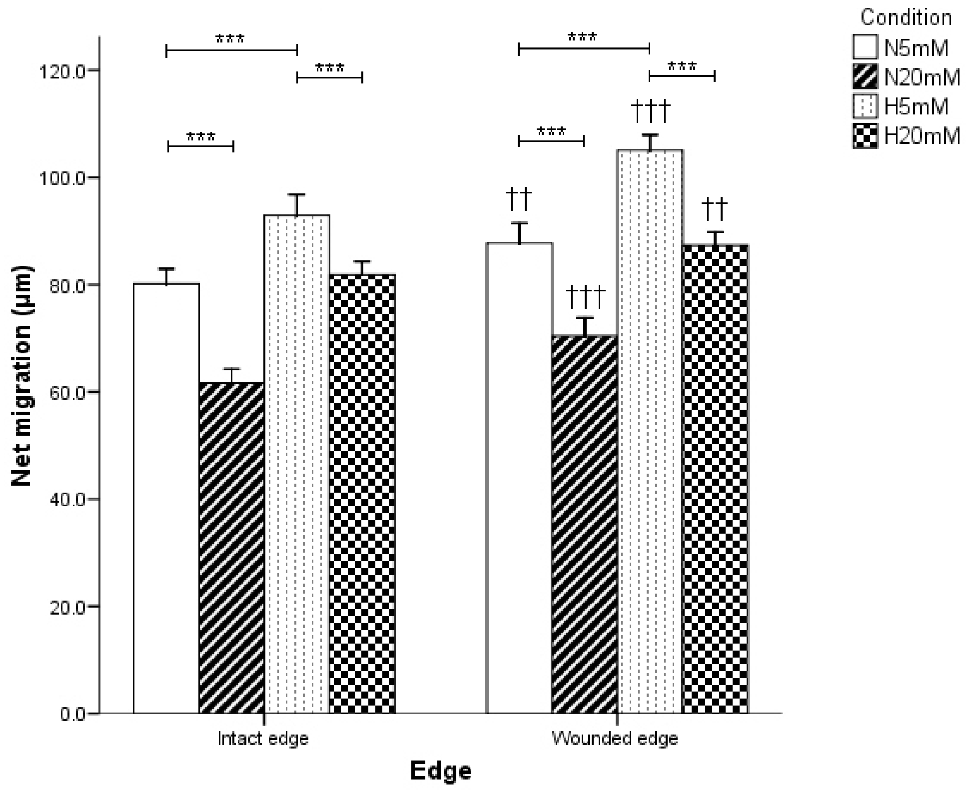
Effect of mannitol



Summary
Hypoxia increased net migration distance at 24 & 48 h.

Increased **mannitol concentration** did not show any significant change in the net migration of endothelial cells at 24 & 48 hours.

Effect of wounding cells



24 h

Summary

Hypoxia increased migration and an increased glucose concentration decreased the net migration. Cells from the wounded edge travelling at a significantly greater distance than cells from intact edge.

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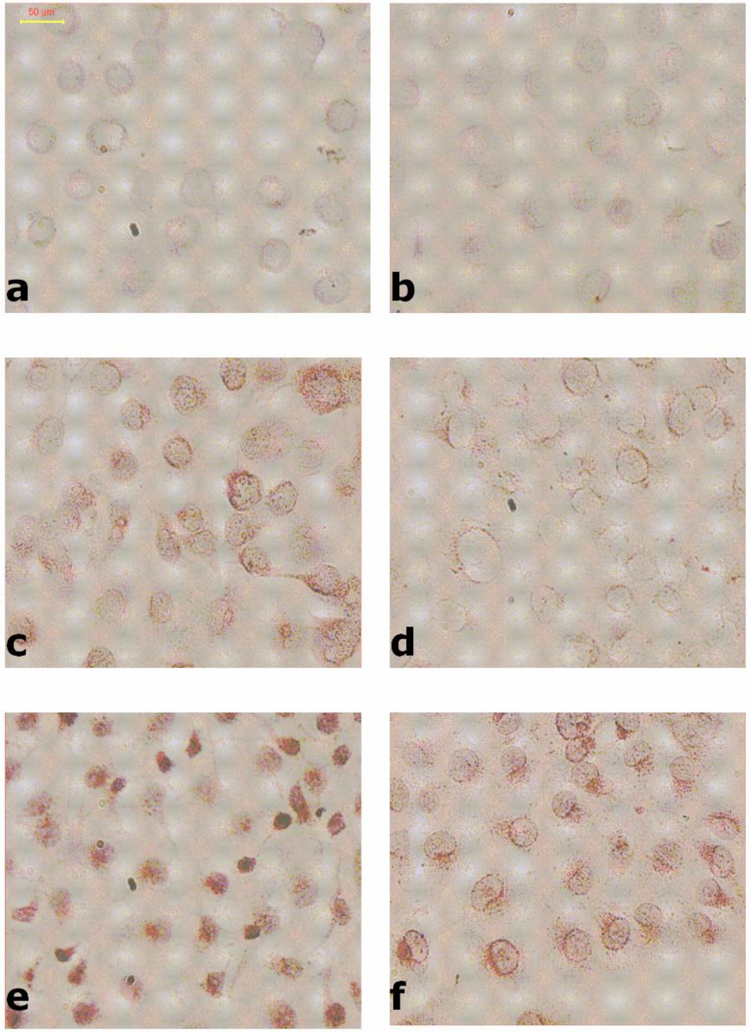
Immunostaining: HIF1α

5mM 20mM

Negative control

normoxia

hypoxia



HUVEC

Hector, MacMannus & Knott (2004)

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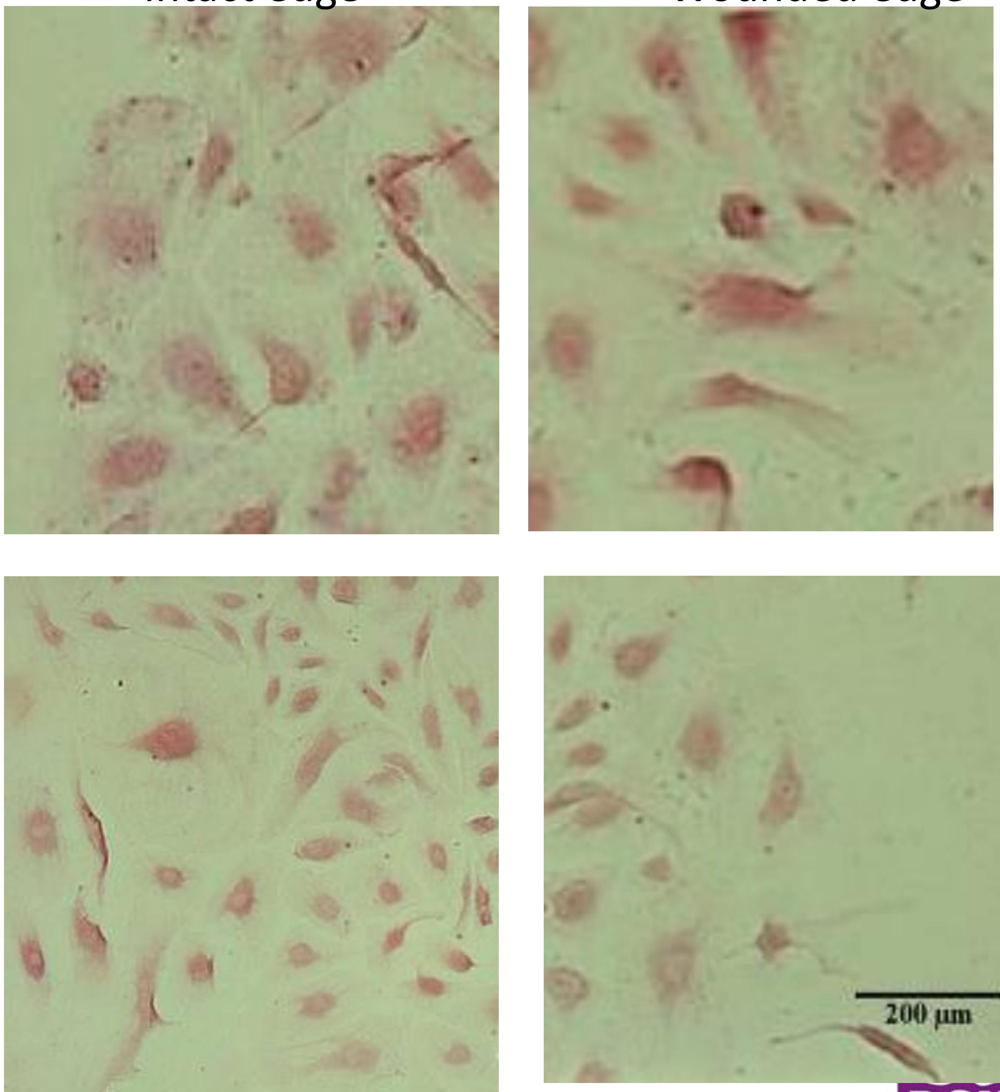
Immunostaining: HIF1α

Intact edge

Wounded edge



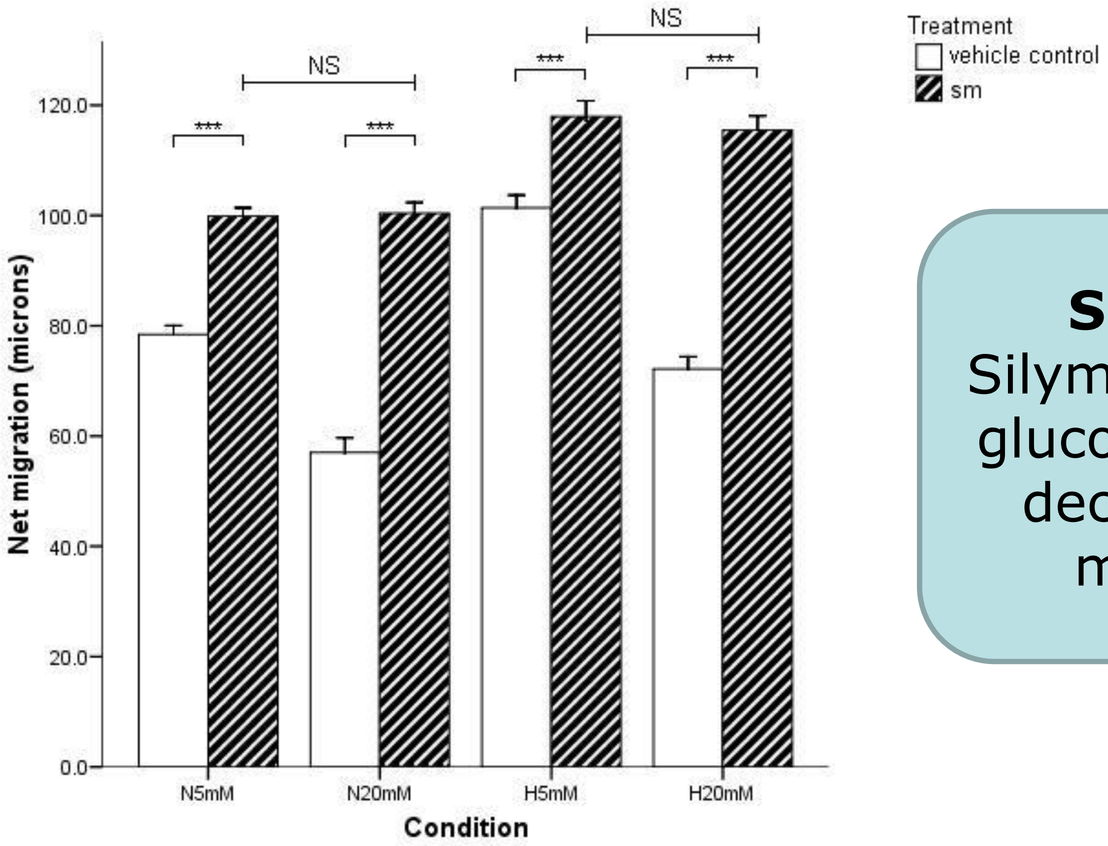
5 mM glucose hypoxia

20 mM glucose hypoxia

HuMVDEC

Reactive oxygen species

- Silymarin
  - added in liquid form



Summary
Silymarin restores glucose mediated decreased cell migration.

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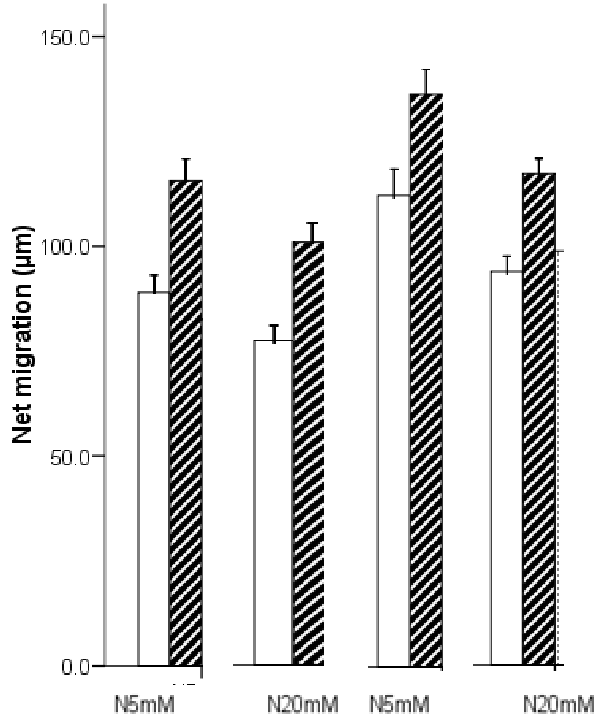
- **Silymarin**
  - Formulated with lyophilised wafers for topical application



normoxia hypoxia

Summary
Silymarin can be incorporated into a lyophilised wafer for topical application to recalcitrant wounds.

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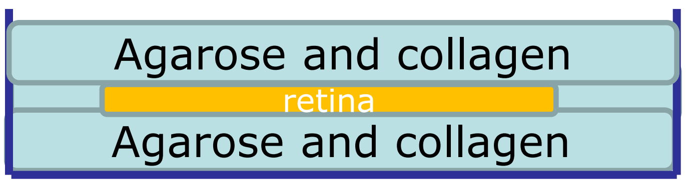
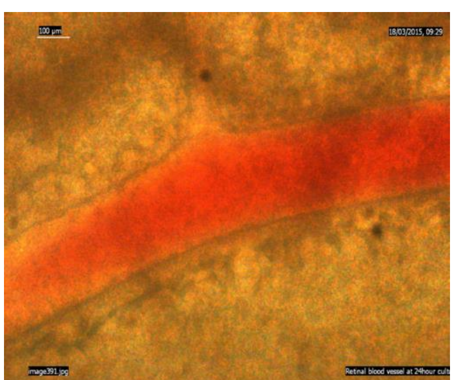
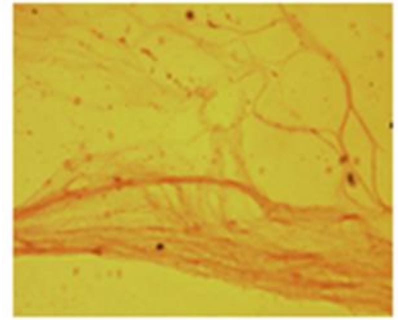
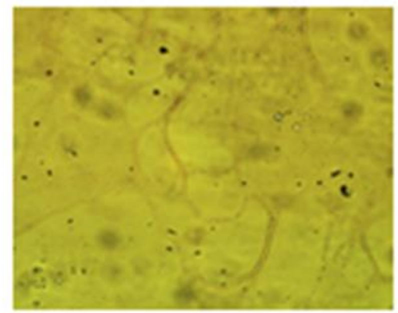
Retinal explant model







Agarose + Collagen



Agarose

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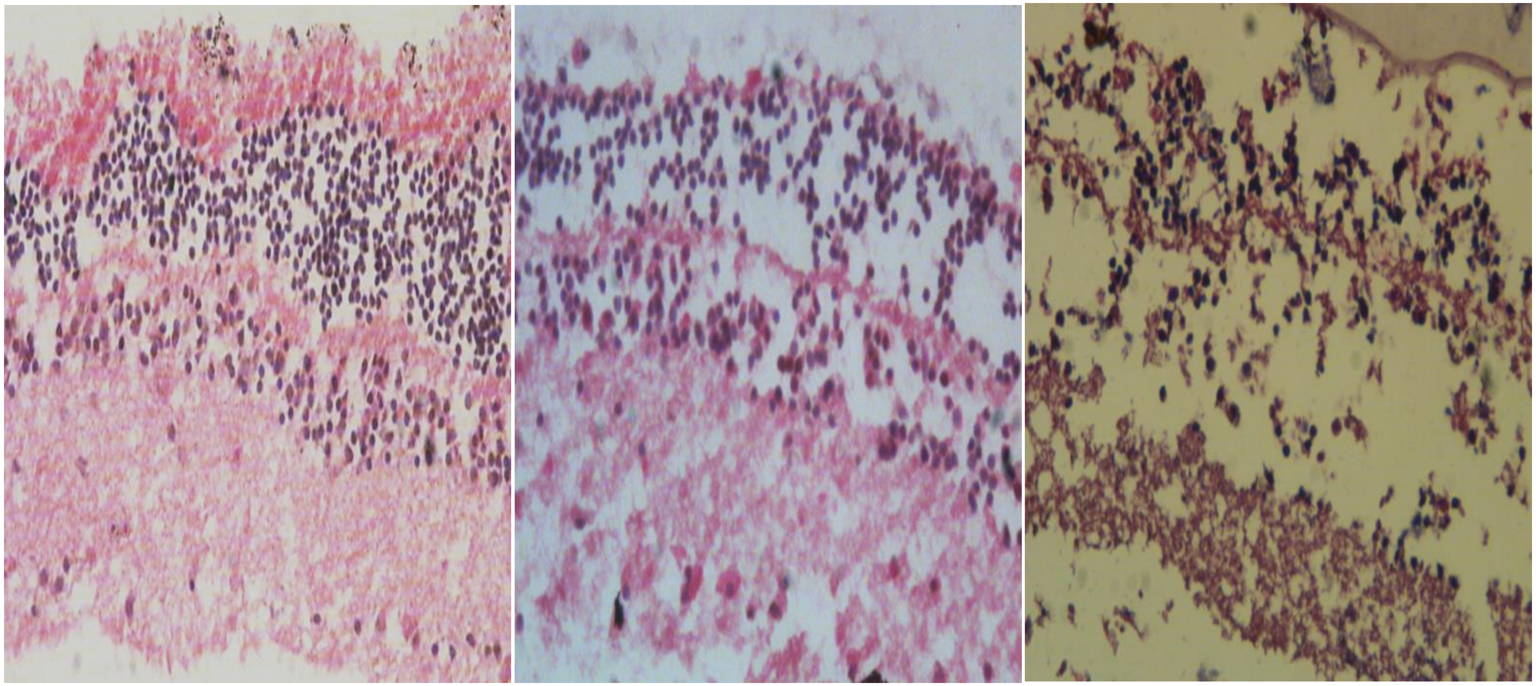
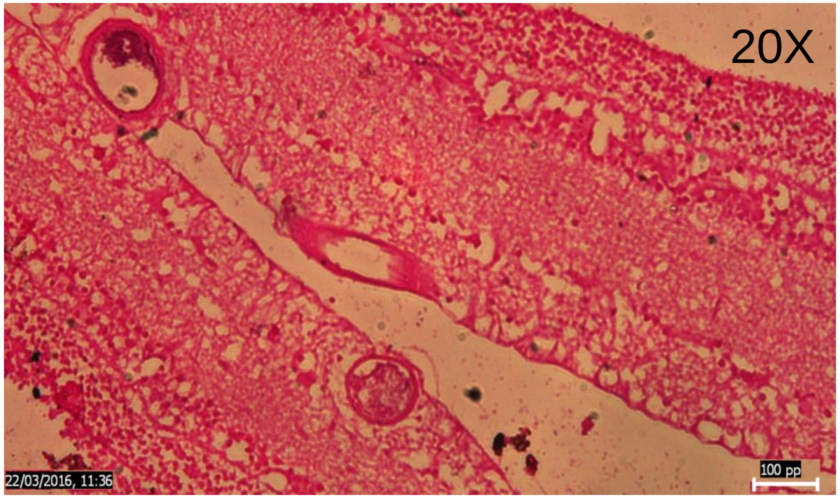
Haematoxylin and eosin staining of retinal explant

- Model shows retinal structural changes

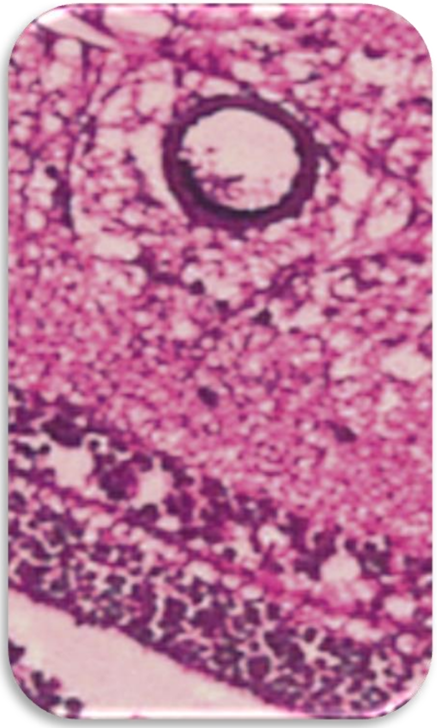


Immunohistochemistry (HIF1α) of retinal explant

PBS



HIF-1α



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Hyperbaric oxygen therapy

What is Hyperbaric Oxygen Therapy (HBOT)?

Hyperbaric oxygen therapy (HBOT) is done in a sealed chamber pressurized at 1 ½ to 3 times normal atmospheric pressure where the patient is breathing pure oxygen.

American Cancer Society. (2010). Hyperbaric Oxygen therapy. Retrieved October 22, 2010 from http://www.cancer.org/Treatment/TreatmentsandSideEffects/ComplementaryandAlternativeMedicine/HerbsVitaminsandMinerals/hyperbaric-oxygen-therapy

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Treatment of recalcitrant ulcers

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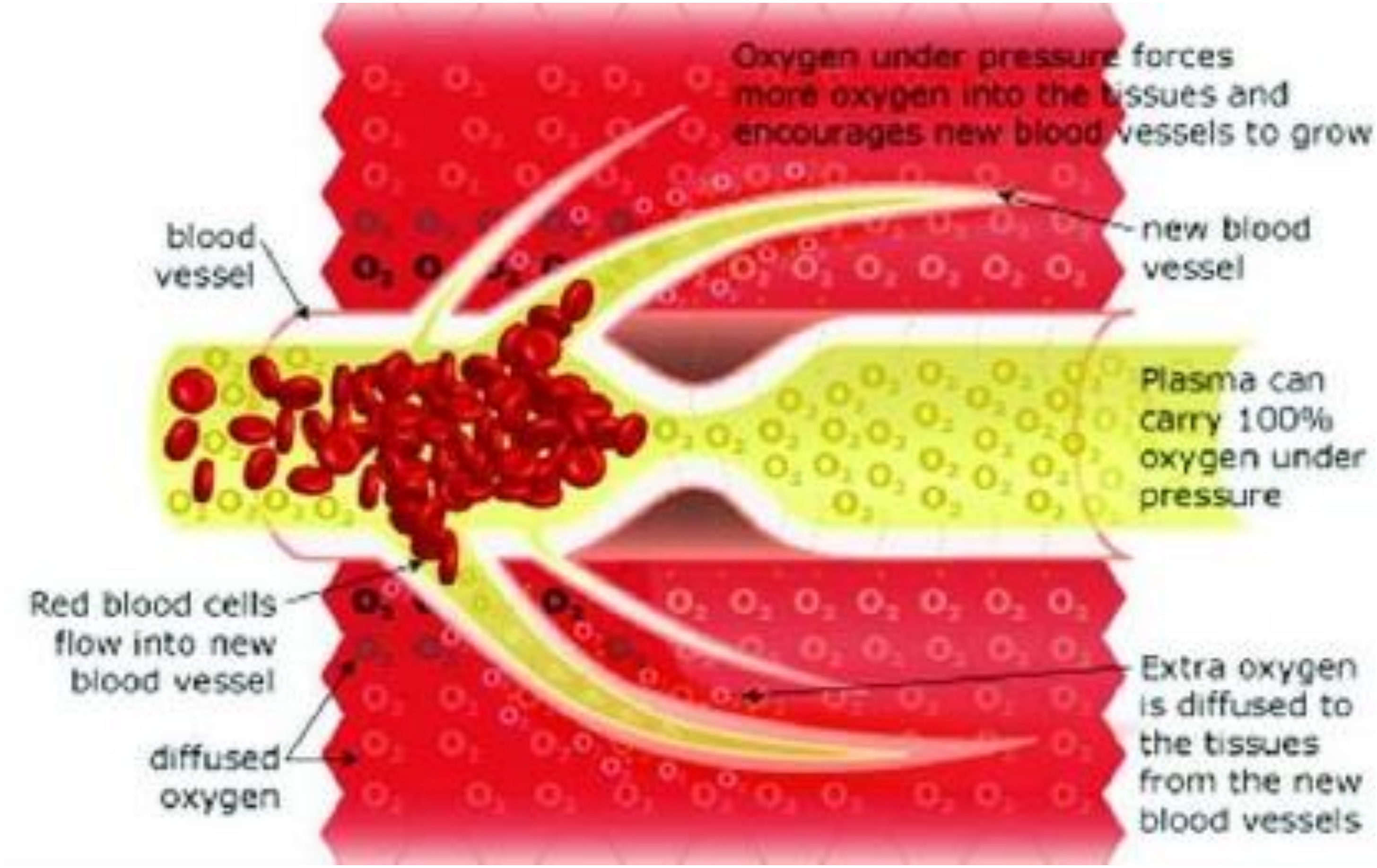
Mechanism of action

- O2 carried by erythrocytes
- HBOT increases O2 solubility
- Crosses cell membranes entering bodily fluids
  - Plasma, lymphatic system, interstitial fluid, cerebrospinal fluid
- Toxicity
  - Lungs (oedema)
  - CNS toxicity (grand mal)
  - Eyes (myopia)

Capillary growth - angiogenesis



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HBOT and hyperoxia

Treatment of recalcitrant ulcers

- Summary
  - Greater understanding of mechanisms of vascular disease
  - Facilitates development in management of diabetes
  - Provides opportunities for continuing development of therapeutic options

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