

Bioengineering Approaches for Enhancing Therapeutic Efficacy of Cell Therapy

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EDUCATION

Ph.D. Dept. of **Chemical Engineering**, Natl. Tsing Hua Univ. (2015)

M.S. Dept. of **Chemical Engineering**, Natl. Tsing Hua Univ. (2010)

B.S. Dept. of **Life Science**, Natl. Tsing Hua Univ. (2008)

WORD EXPERIENCE

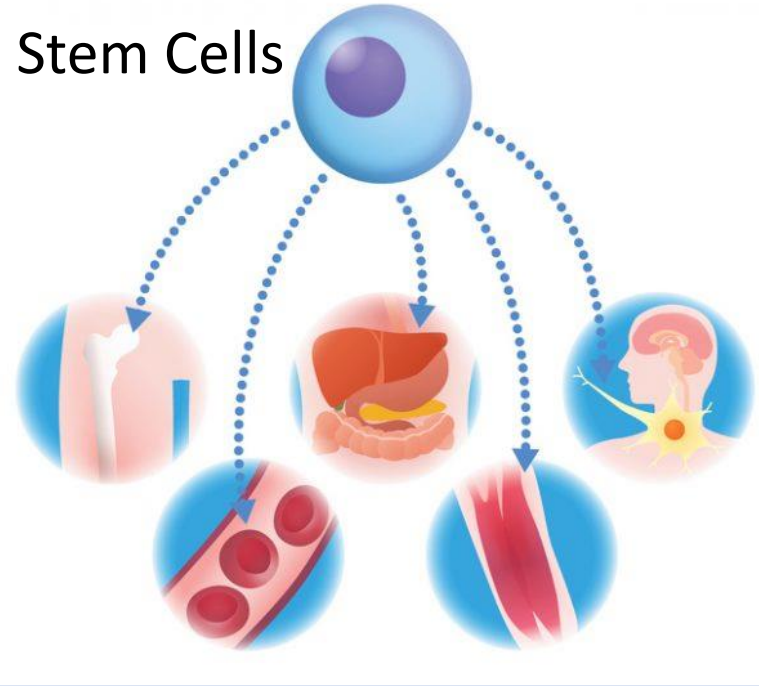
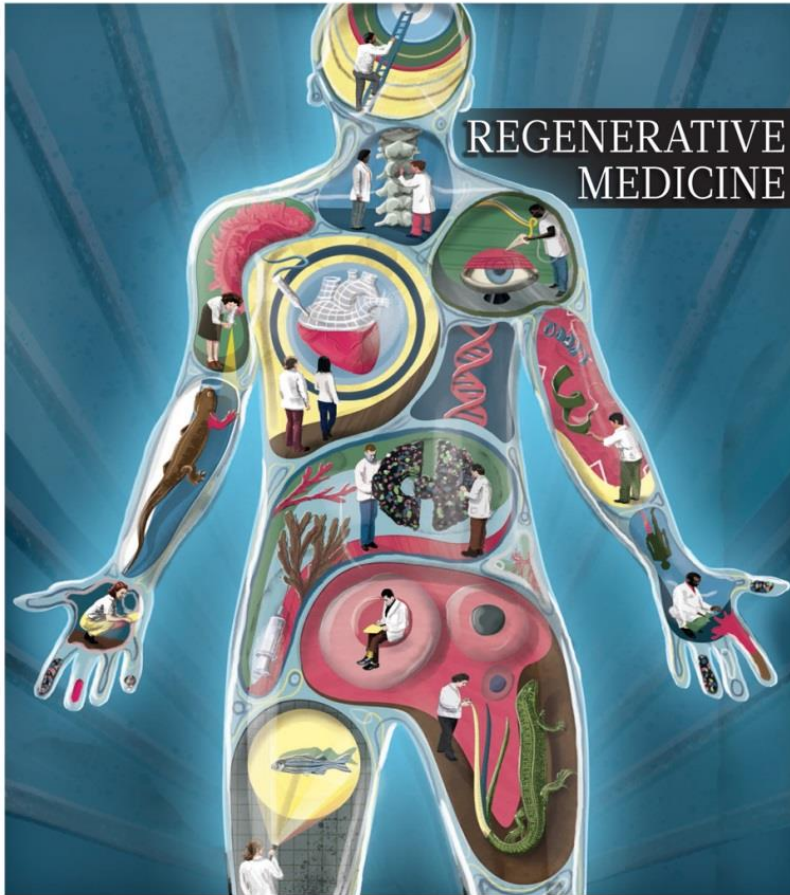
2017/2 – present **Assistant Professor**

Institute of Biomedical Eng., Natl. Tsing Hua Univ.

2015/8 – 2017/1 Postdoctoral Fellow

Dept. of Chemical Eng., Natl. Tsing Hua Univ.

Div. of Cardiac Surgery, Univ. of Toronto, Canada

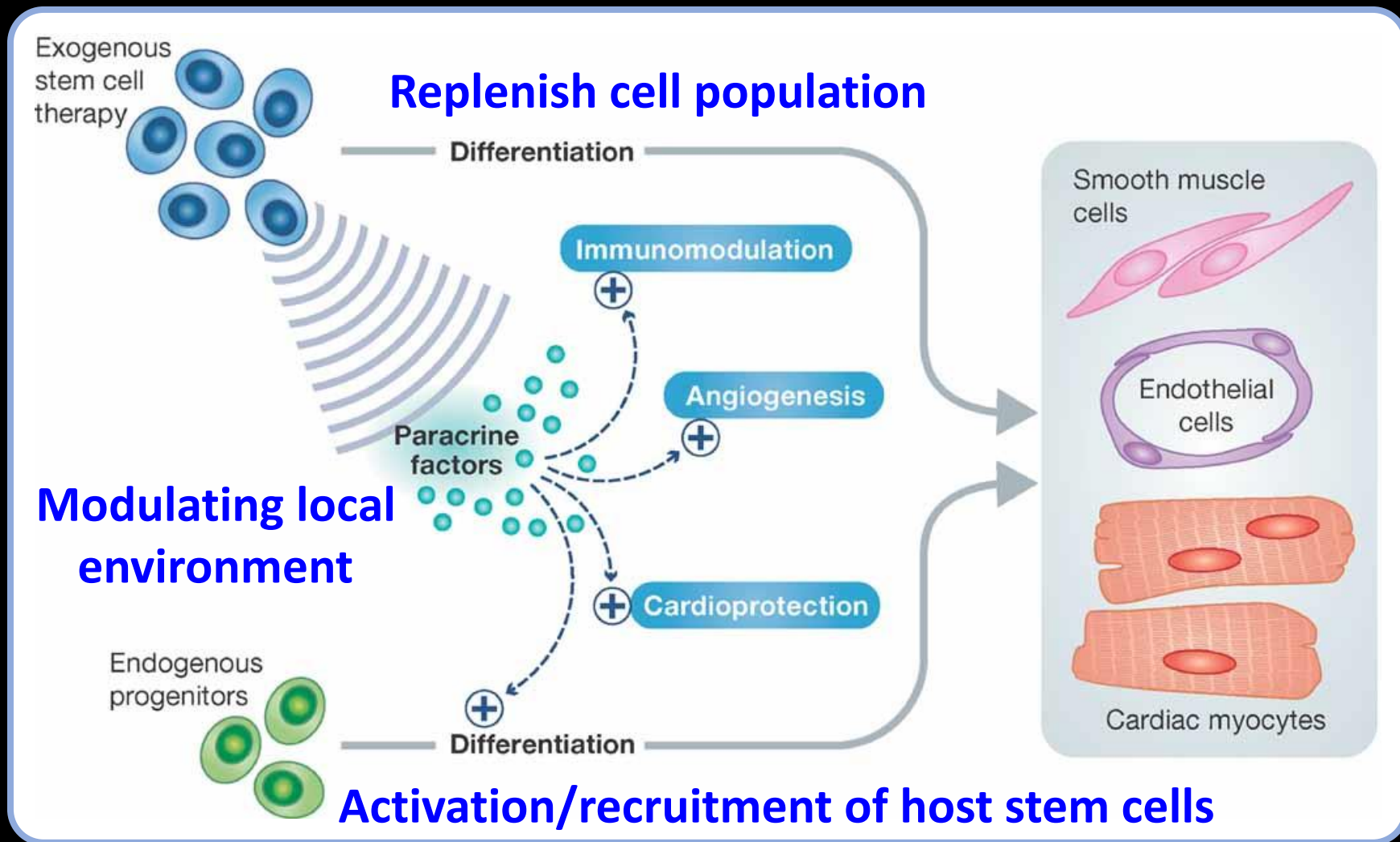


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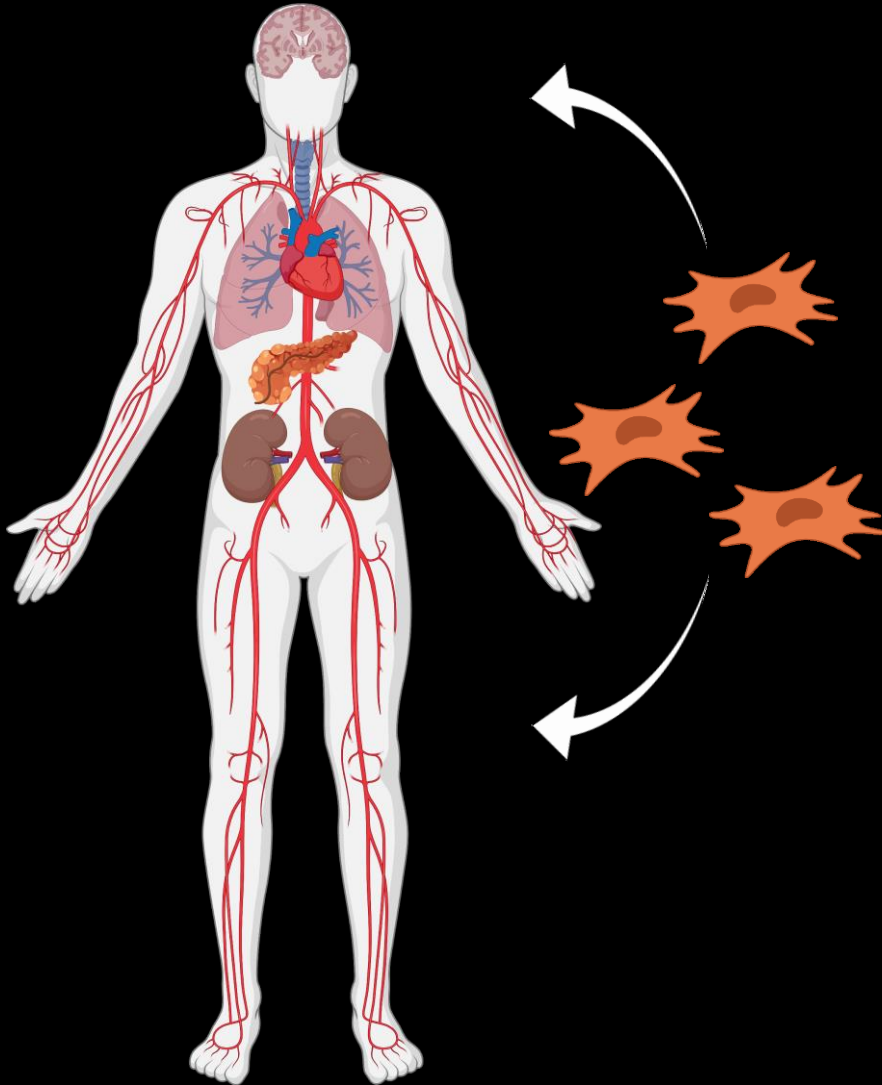


Rebuilding
the body

Stem Cell-based Therapy



Cell-based Therapy



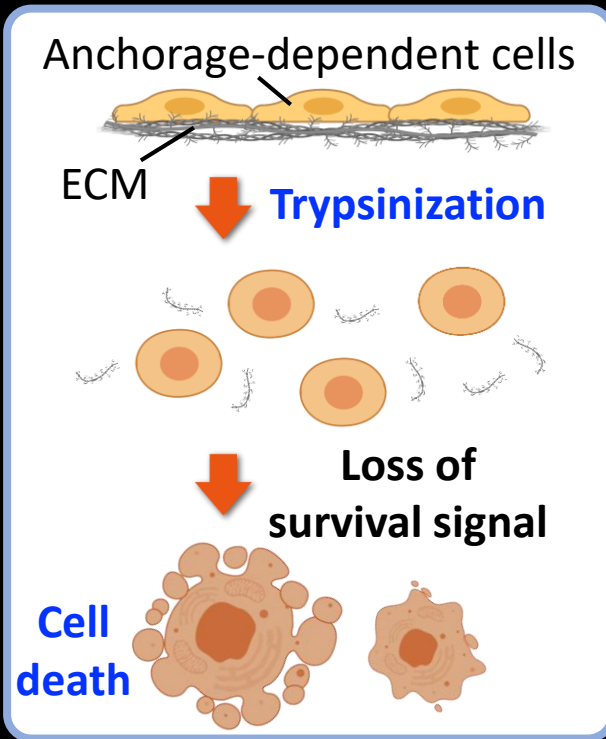
Transplanted cells must be able to:

- 1. remain at target site**
- 2. survive**

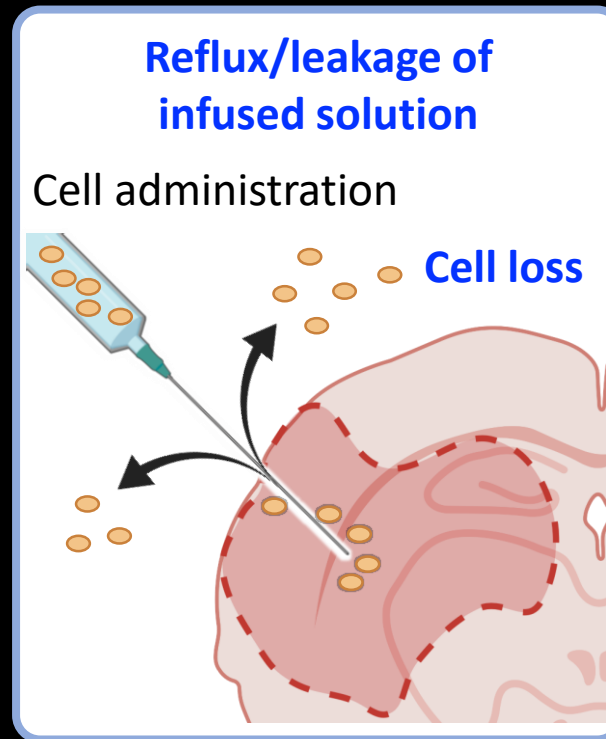
to eventually exert their therapeutic function

Current Limitation of Cell Therapy

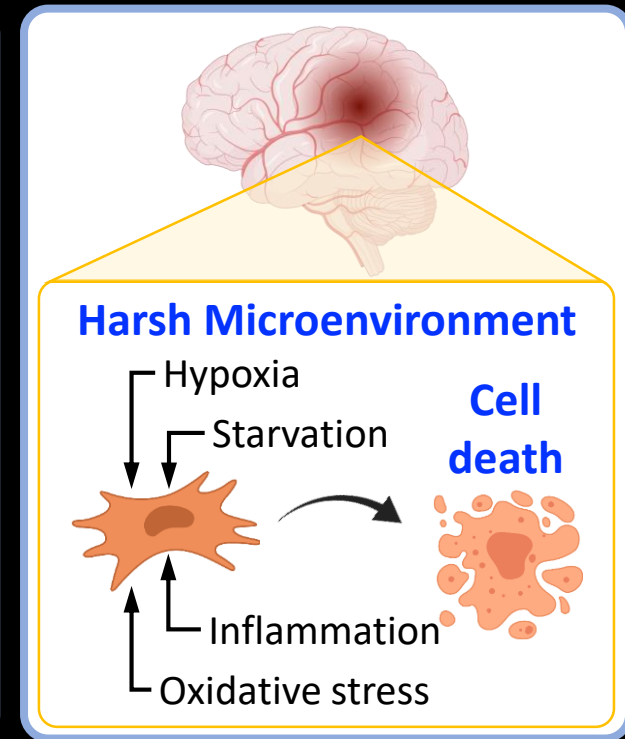
Anoikis



Cell dispersal

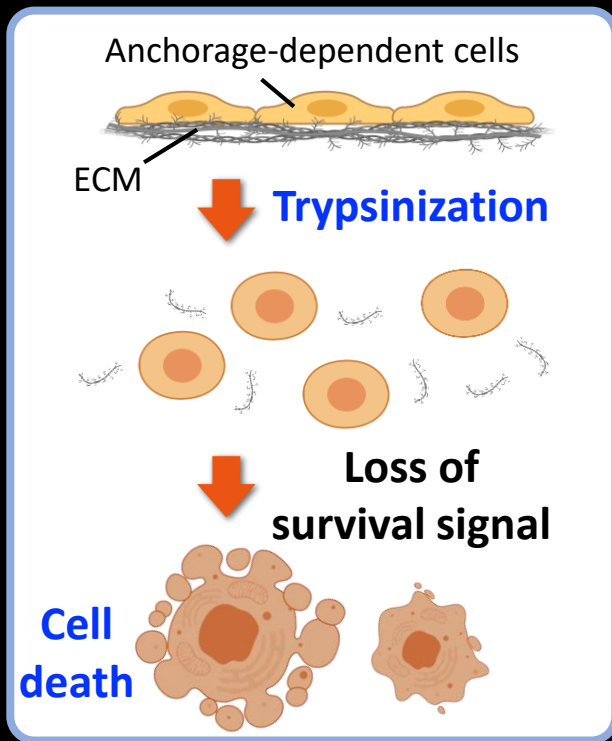


Hostile target tissue



Current Limitation of Cell Therapy

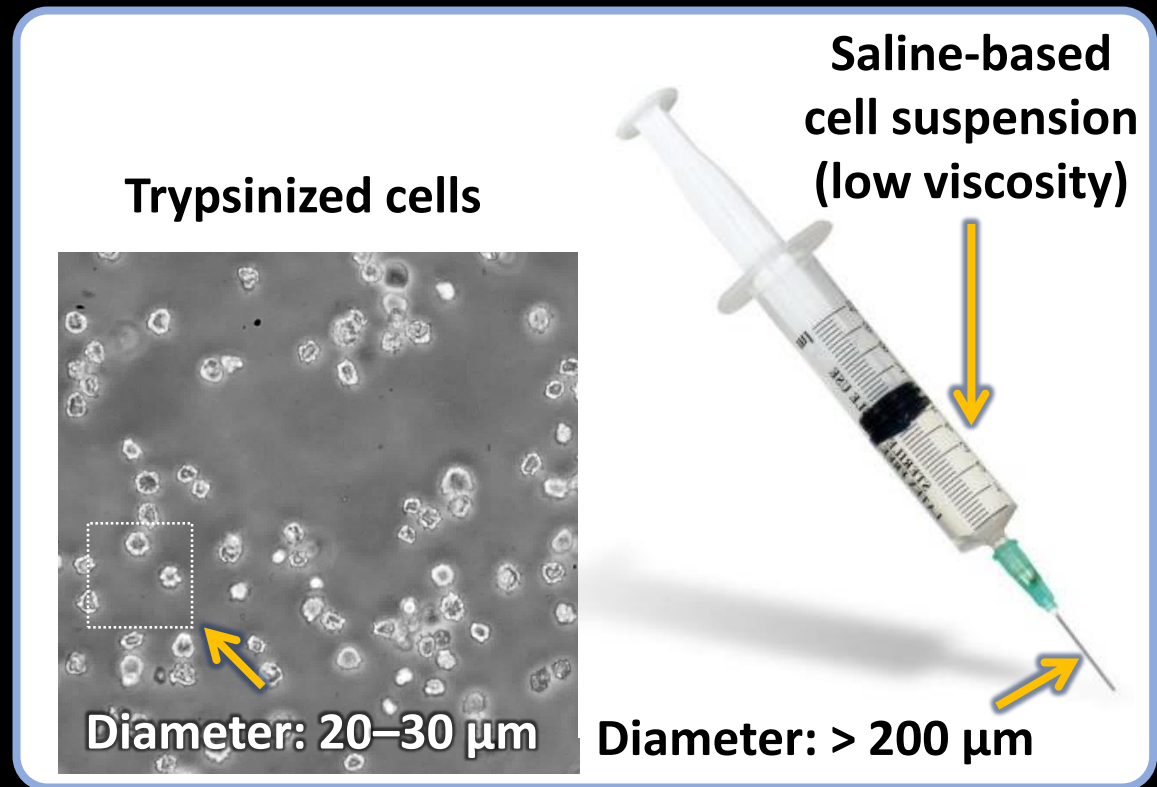
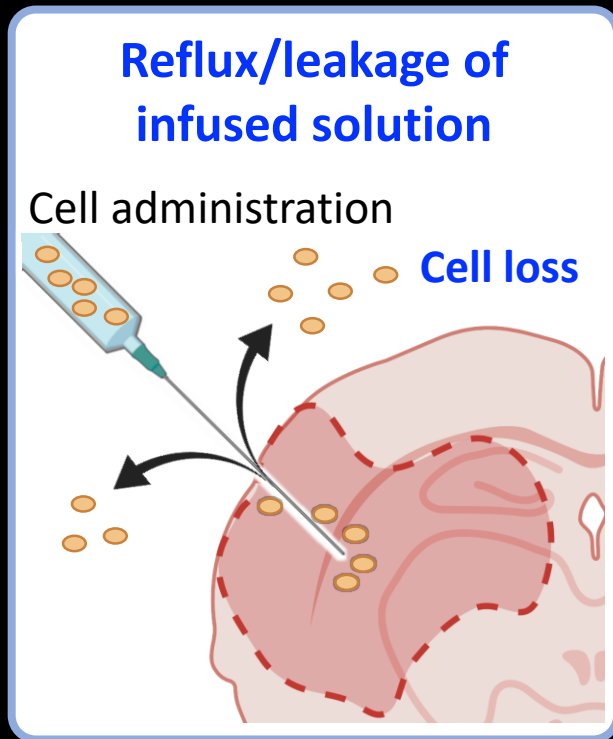
Anoikis



- Proteolytic enzymes disrupt **cell-cell & cell-ECM** interaction
- **Anoikis**: Programmed cell death that occurs in **anchorage-dependent cells** when they detach from surrounding ECM

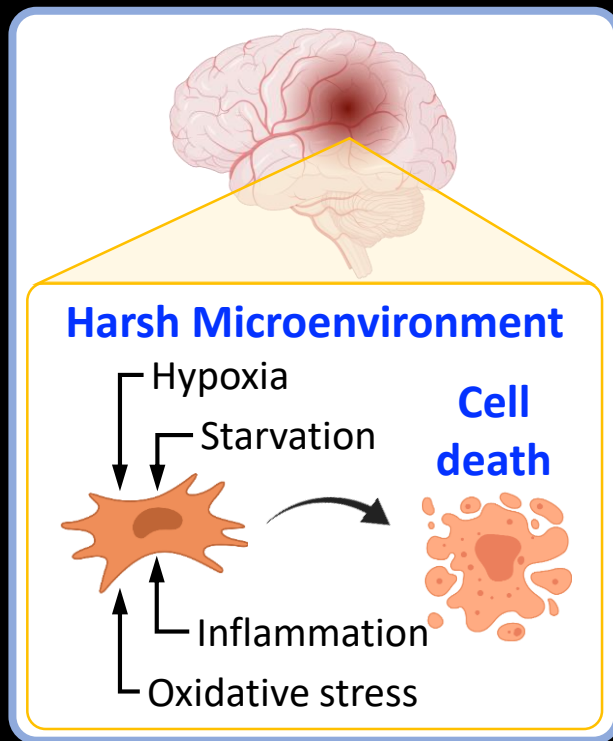
Current Limitation of Cell Therapy

Cell dispersal



Current Limitation of Cell Therapy

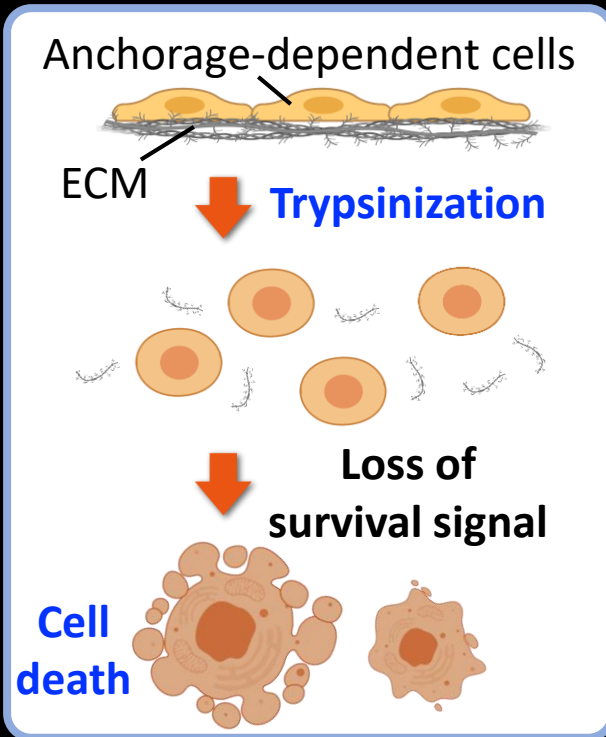
Hostile target tissue



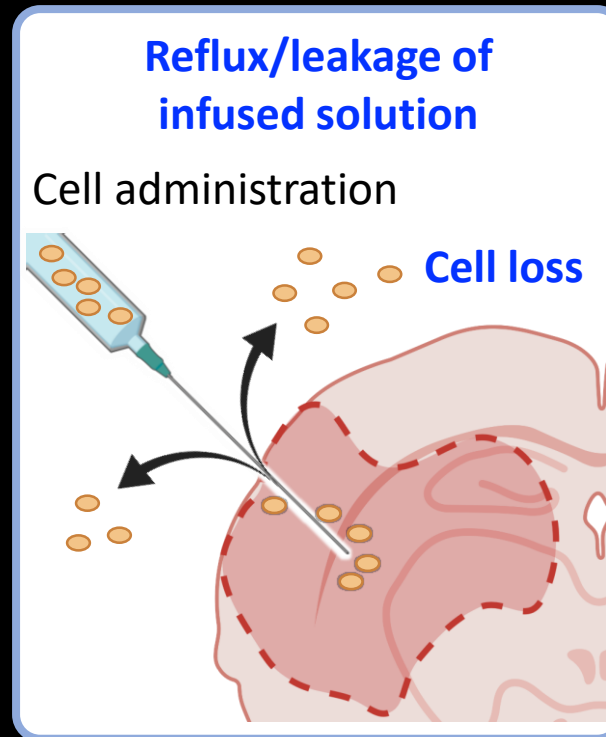
- **Harsh environment** in target tissue
- **Ischemia, hypoxia, inflammation, oxidative stress, ionic imbalance**

Current Limitation of Cell Therapy

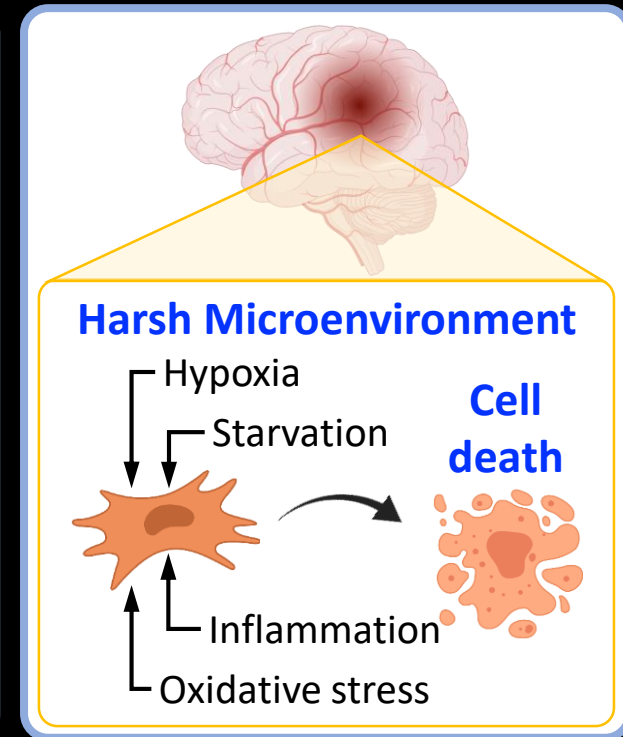
Anoikis



Cell dispersal



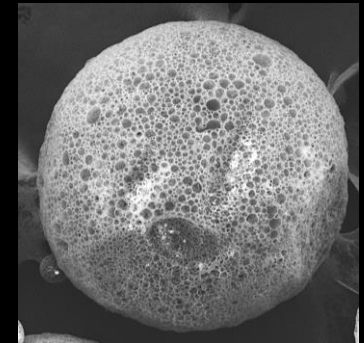
Hostile target tissue



- Inadequate **retention & survival** of engrafted cells
- Limited **therapeutic efficacy**

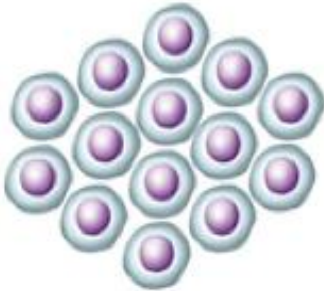
Enhancing Therapeutic Efficacy of Cell Therapy

1. Transplant cells in a **3D spheroid configuration**
2. Modulate the microenvironment of target tissue with **functional biomaterials**



3D Cell Spheroid

Cell Suspension



vs.

3D Cell Spheroid

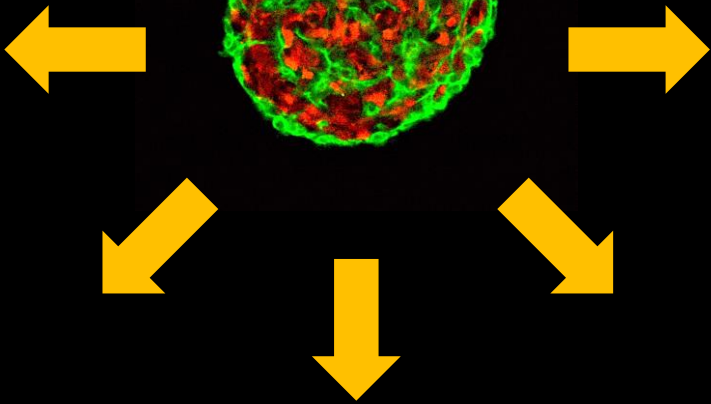
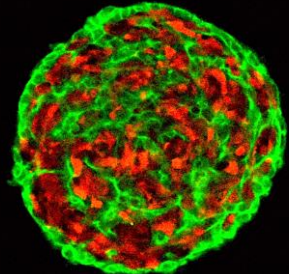


- **Native tissue-mimicking microenvironment**
- **Well preserved cell-cell & cell-ECM interaction**
- **Increased physical volume**
- **Enhanced cell delivery efficiently**
- **Increased therapeutic efficacy**

Cell Therapy Using 3D Stem Cell Spheroids

Ischemic stroke
Traumatic brain injury

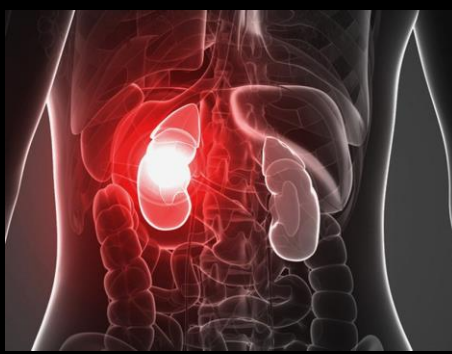
Type I diabetes mellitus



Peripheral nerve injury

Kidney injury

Tendon/ligament injury

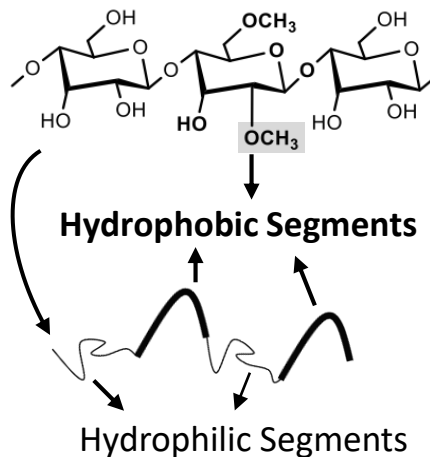


Cell Types for Transplantation

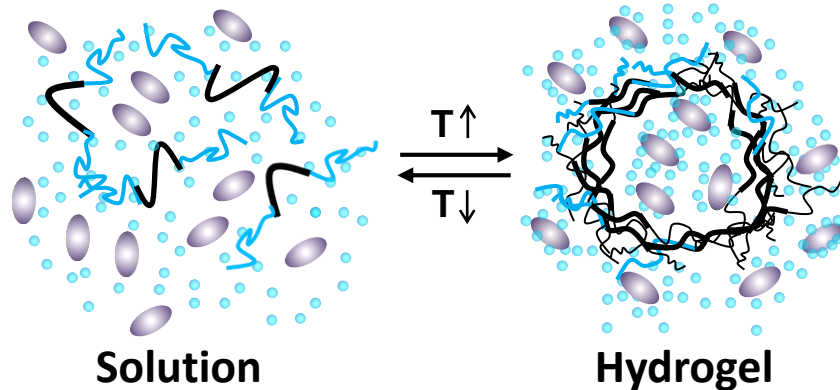
- **Umbilical cord blood mesenchymal stem cell (MSC)**
 - high differentiation potential
 - stored in advance and rapid available
 - no ethical controversy
 - expressing red fluorescent protein (RFP)
- **Human umbilical vein endothelial cell (HUVEC)**

Fabrication of 3D Cell Spheroid

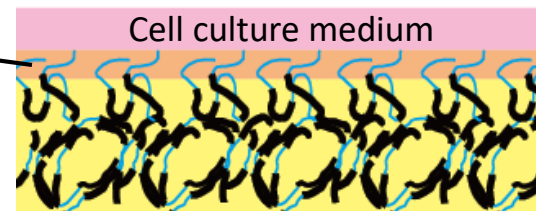
Methylcellulose (MC) Hydrogel



Reversible thermal-responsive gelation



Hydrated Layer (hydrophilic)
Prevent protein adsorption
& cell attachment



Fabrication of 3D Cell Spheroid

Methylcellulose (MC) Hydrogel

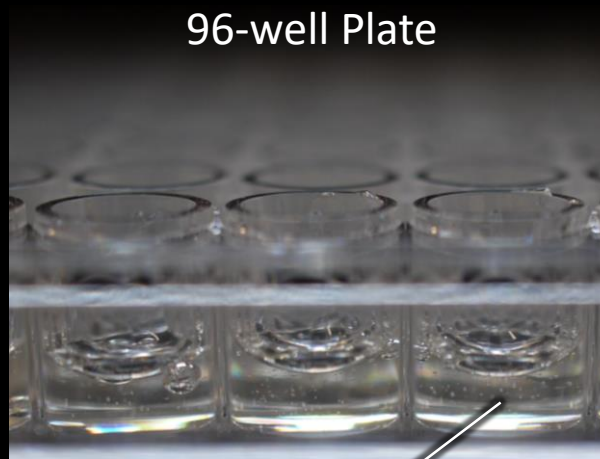
Room
Temperature



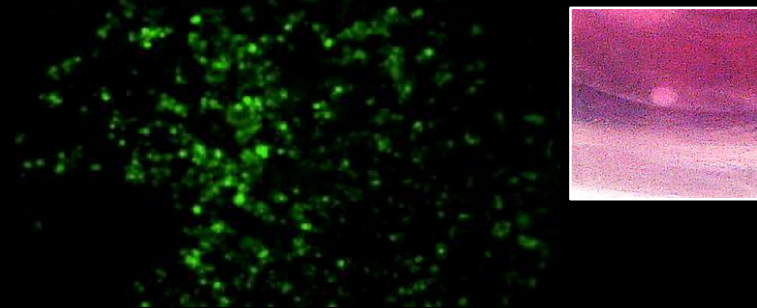
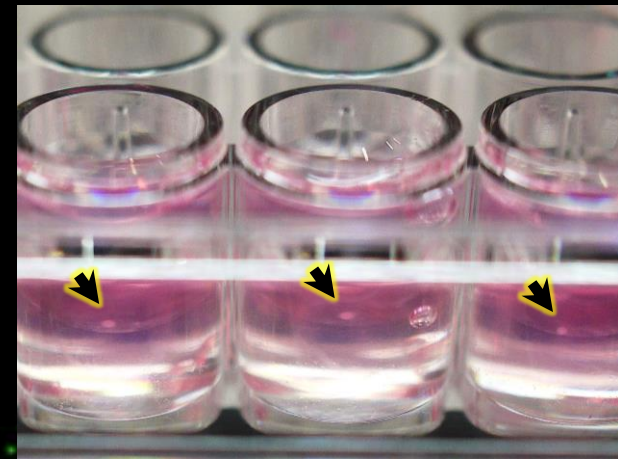
37 °C



96-well Plate

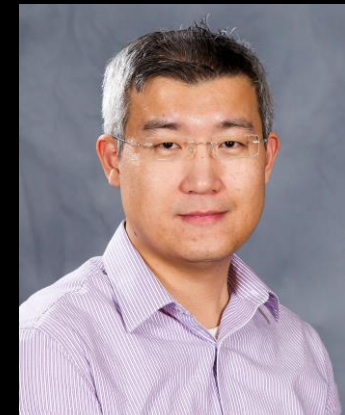
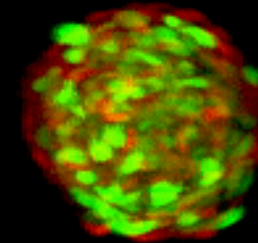
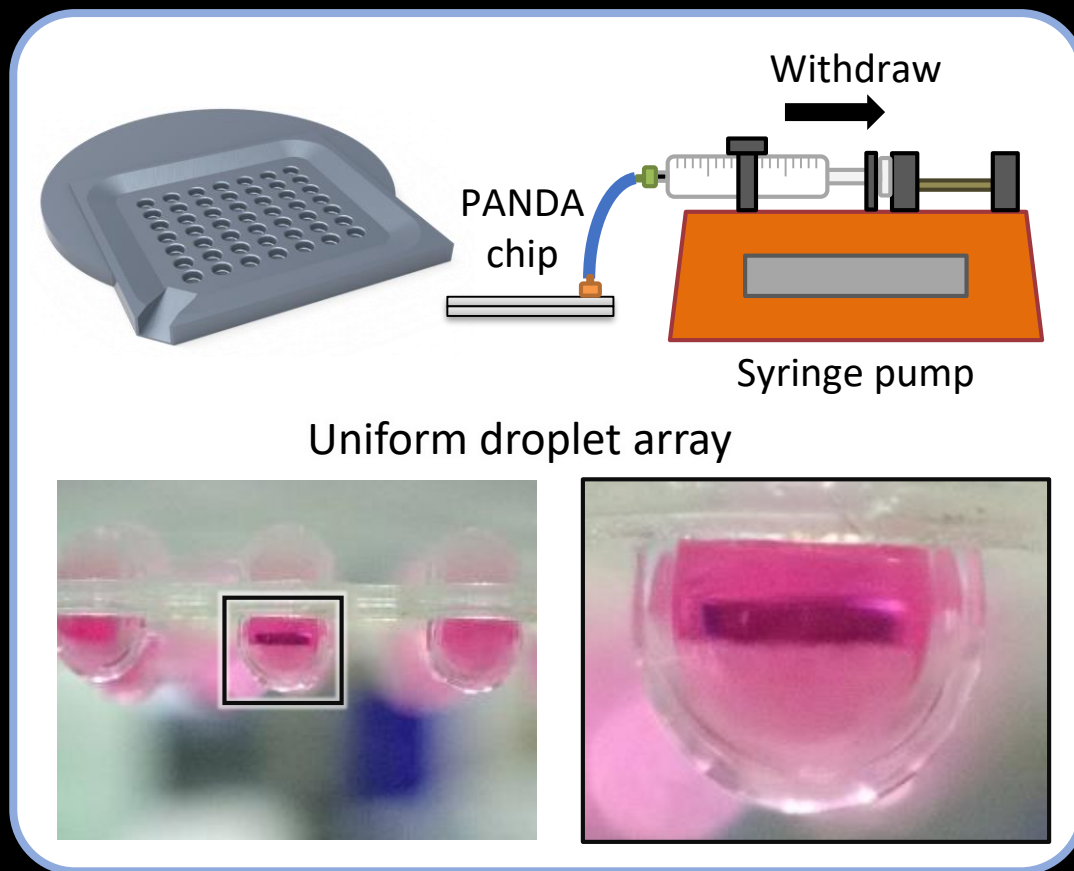


MC Hydrogel



Fabrication of 3D Cell Spheroid

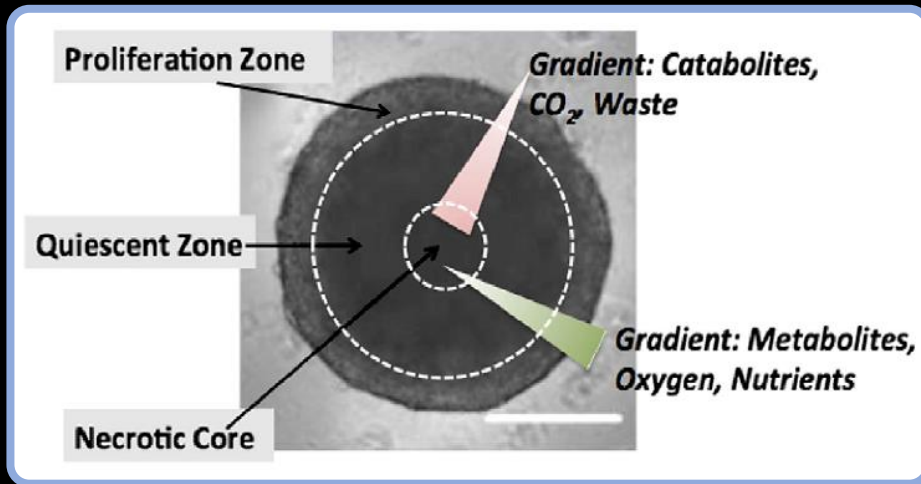
Pressure-assisted network for droplet accumulation (PANDA) chip



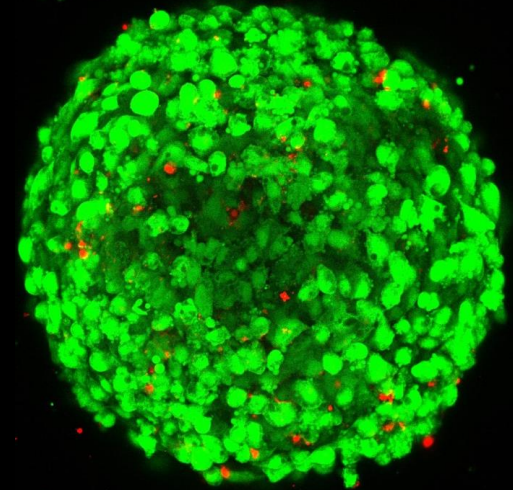
Prof. Jen-Huang Huang
Dept. of Chemical Engineering, NTHU

Fabrication of 3D Cell Spheroid

Cell Viability



10,000 cells/spheroid



Diameter:
300µm

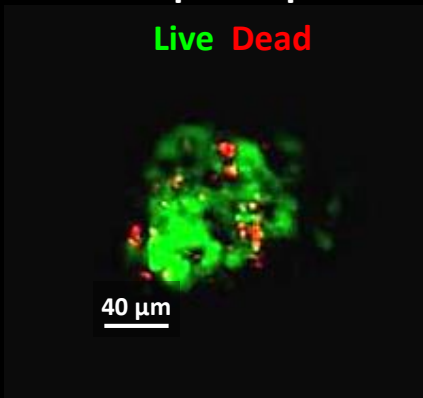
5,000 cells/spheroid

25 µm depth

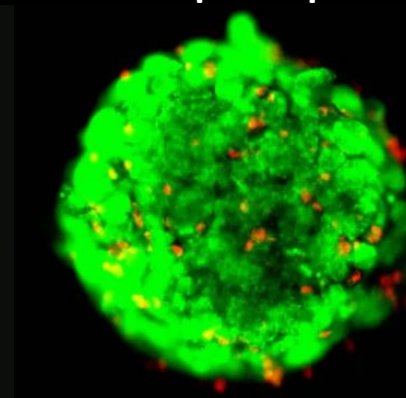
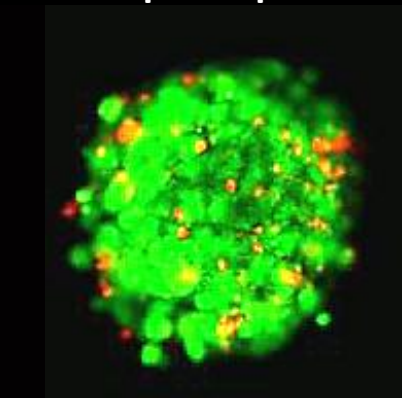
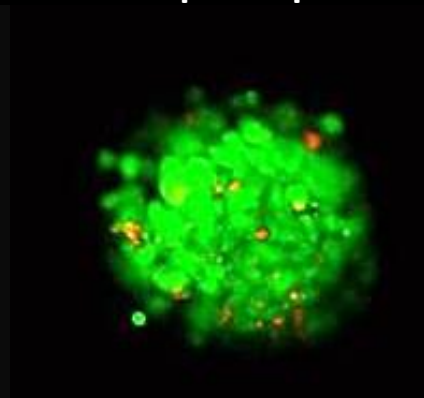
50 µm depth

75 µm depth

100 µm depth



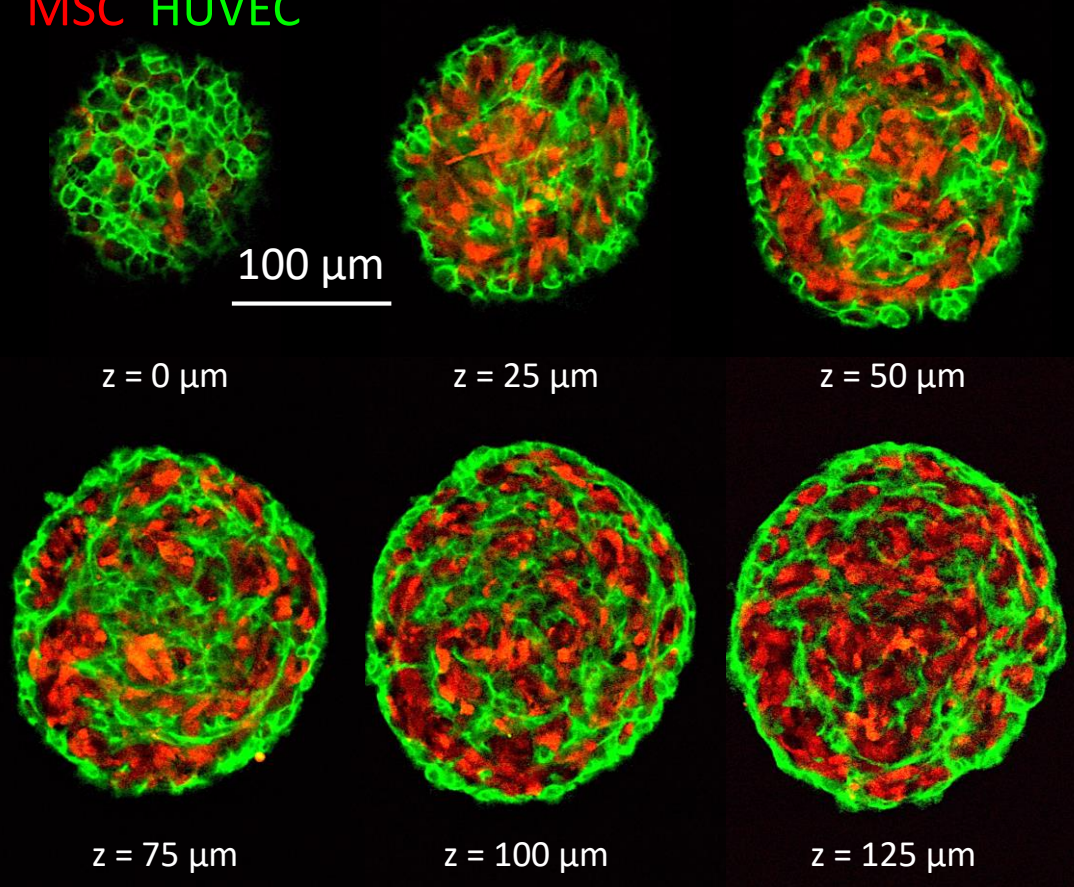
40 µm



Diameter:
200 µm

Fabrication of 3D Cell Spheroid

MSC HUVEC

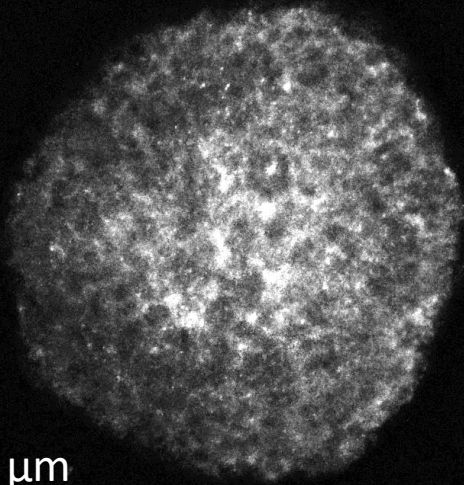


- Intensive cell-cell interaction

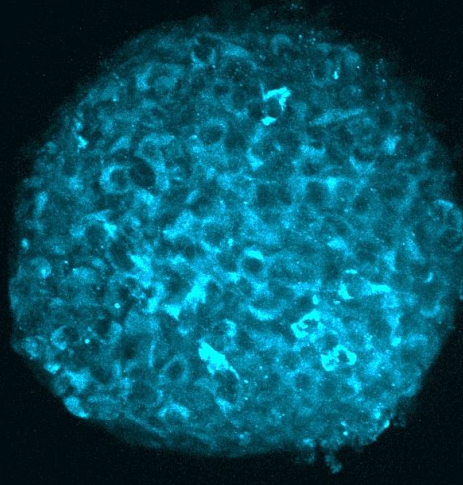
Fabrication of 3D Cell Spheroid

Extracellular Matrix

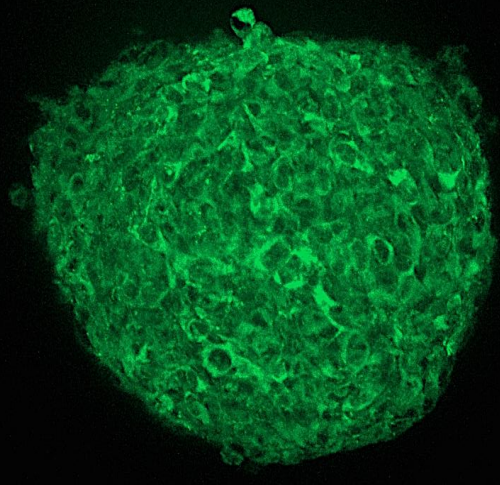
Collagen



Fibronectin



Laminin



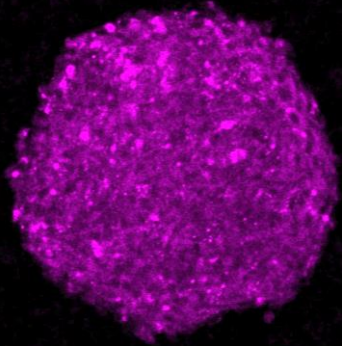
100 μm

- Prevent anoikis before/during transplantation

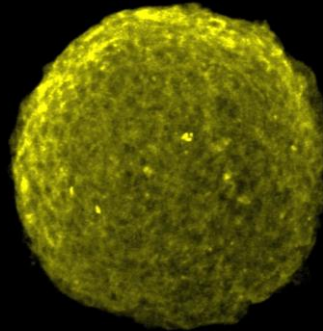
Fabrication of 3D Cell Spheroid

Soluble Factors

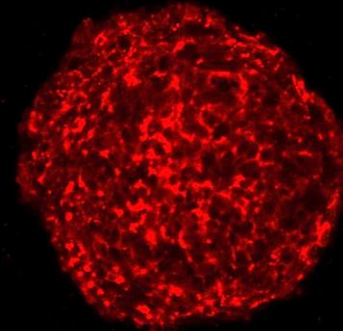
BDNF



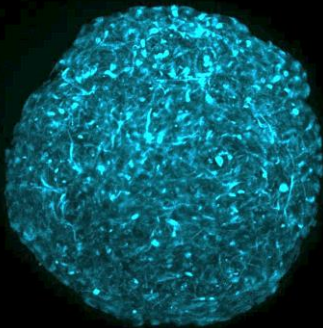
VEGF



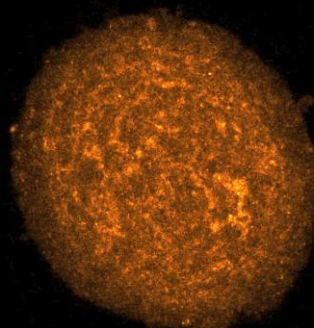
IGF-1



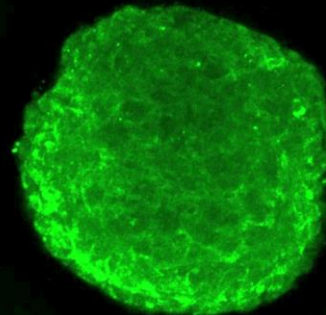
HGF



SDF-1



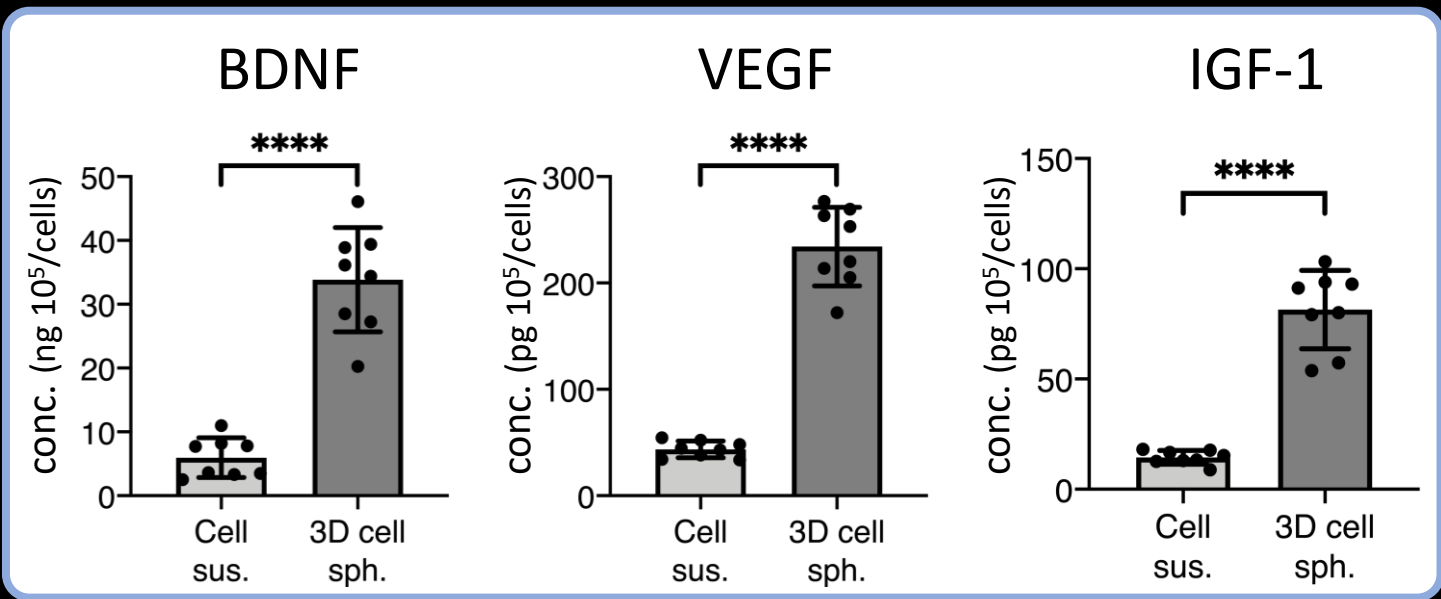
TIMP-3



- Prevent cell survival & functionality

Fabrication of 3D Cell Spheroid

Soluble Factors



- Enhance paracrine secretion & preservation

Therapeutic Potential of 3D Stem Cell Spheroid

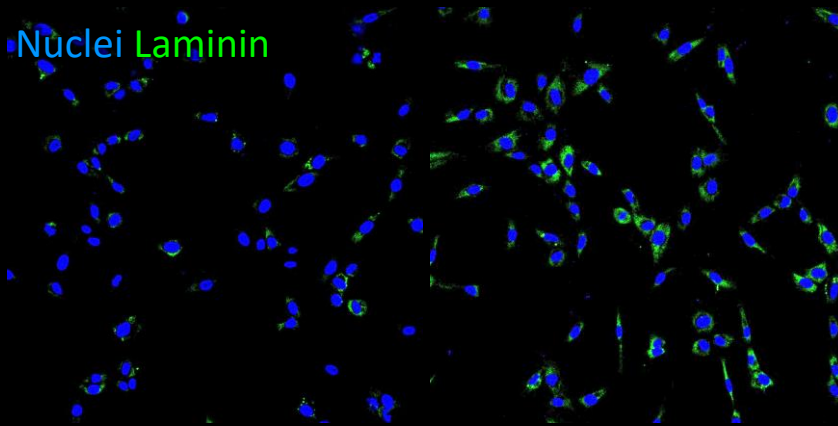
Cell Attachment, Migration & Proliferation

4 h after seeding

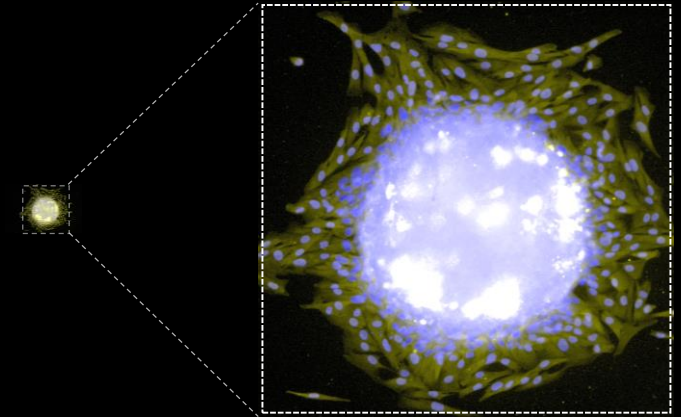
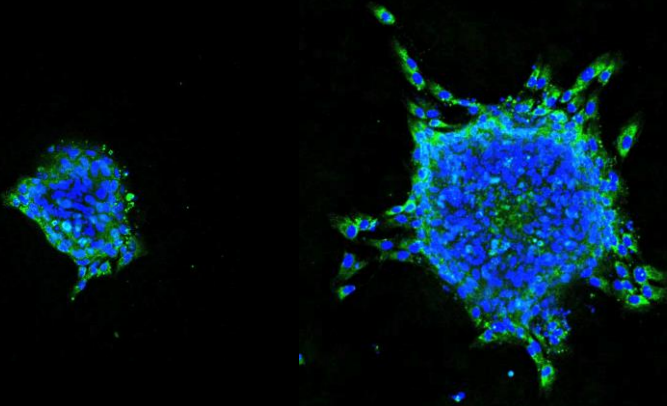
24 h after seeding

Nuclei Laminin

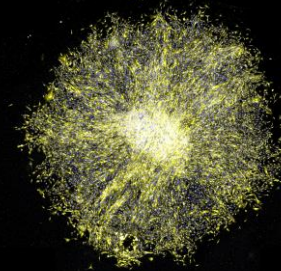
Cell sus.



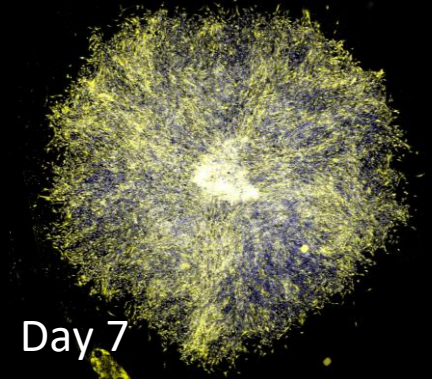
3D cell sph.



Day 1



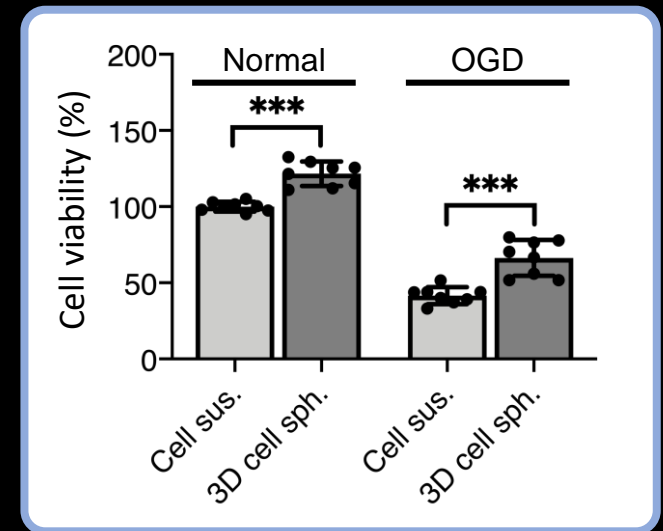
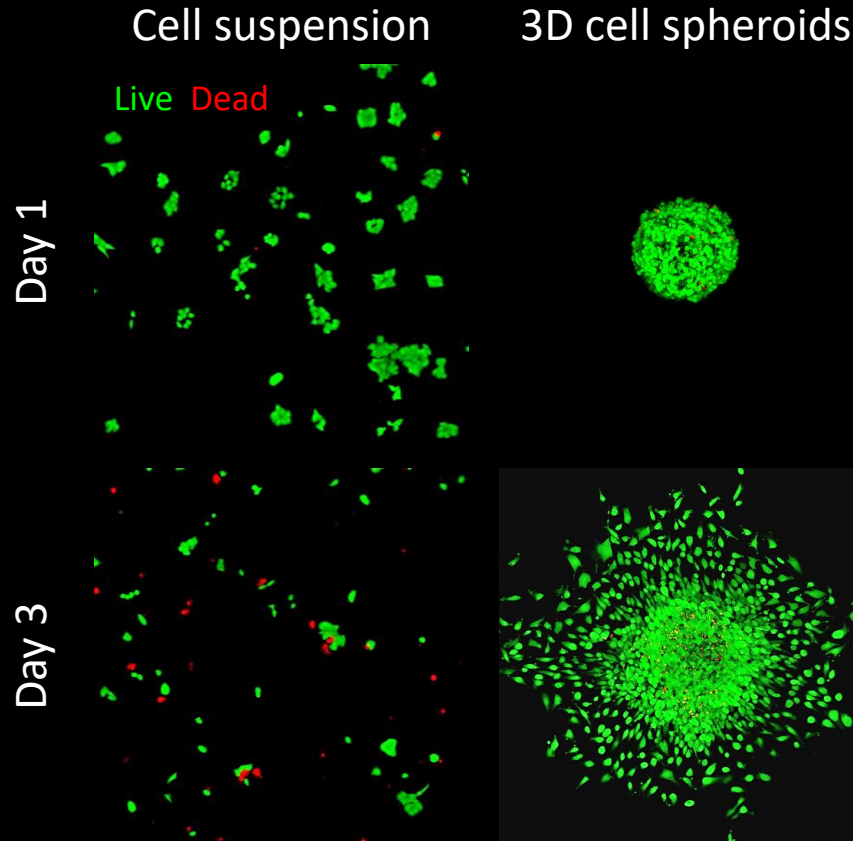
Day 4



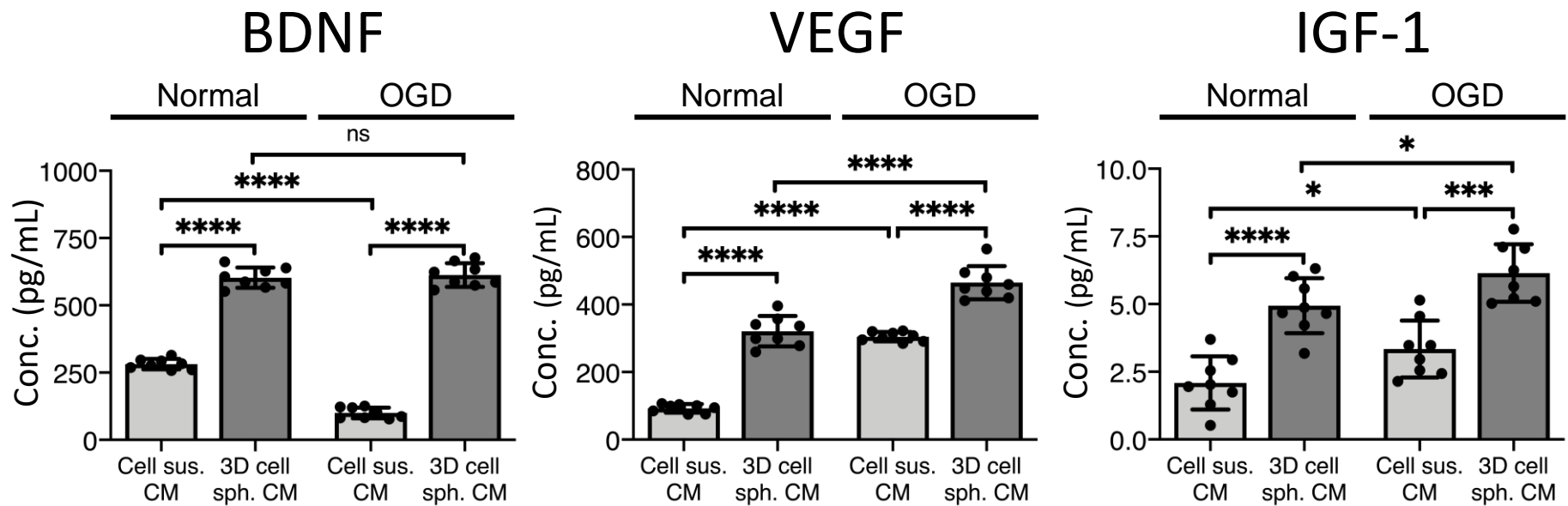
Day 7

Therapeutic Potential of 3D Stem Cell Spheroid

Oxygen & glucose deprivation (OGD):
an *in vitro* model of ischemia

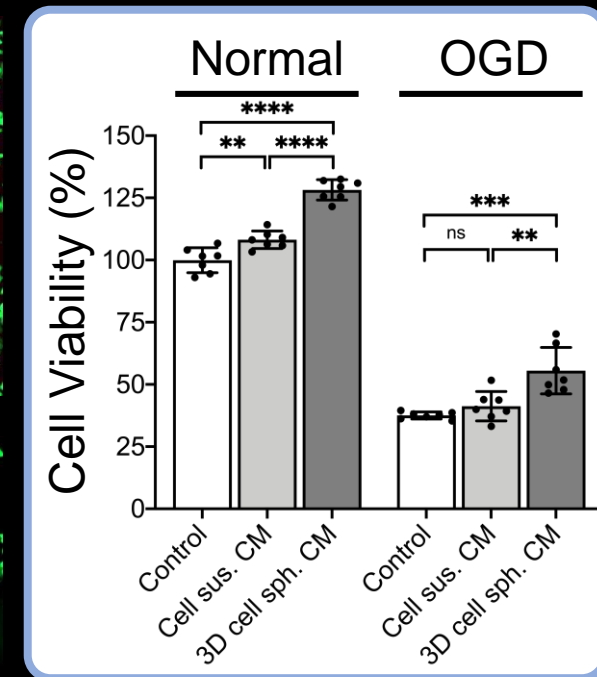
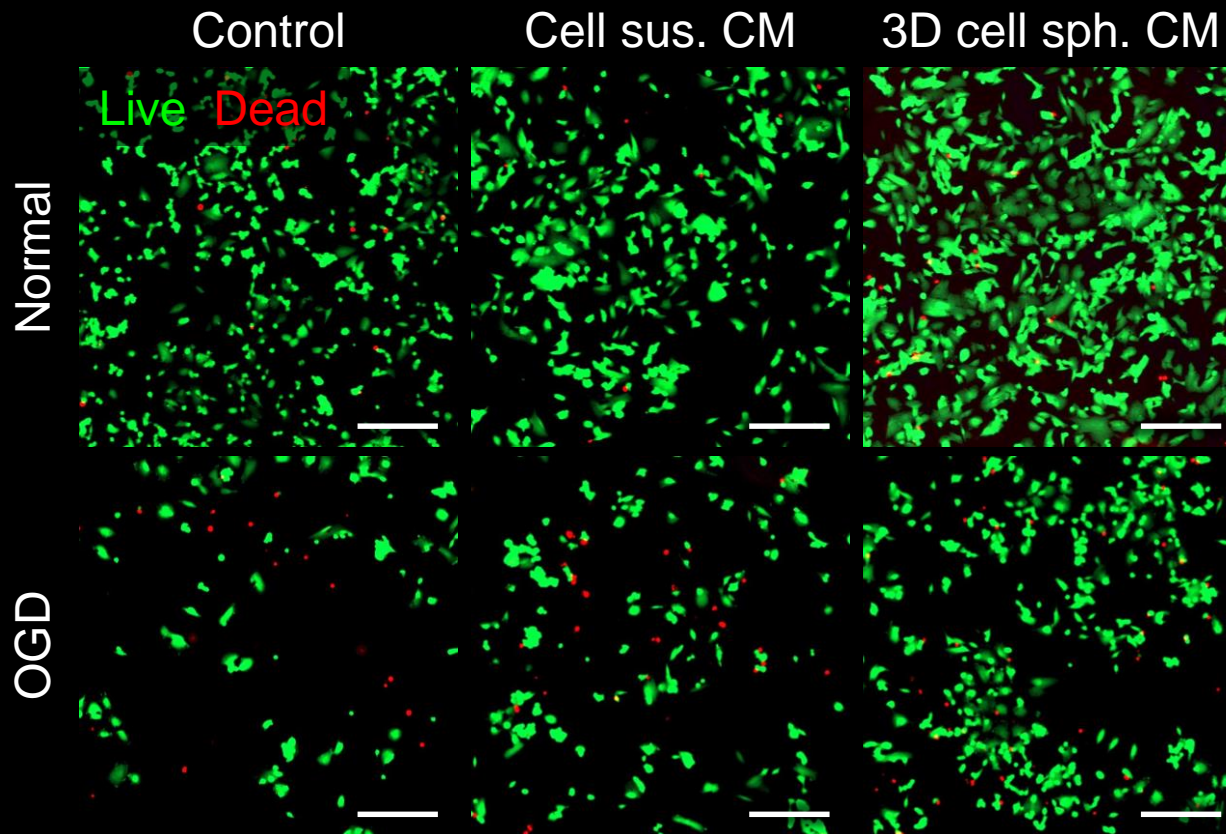


Therapeutic Potential of 3D Stem Cell Spheroid Conditioned Medium (CM)



Therapeutic Potential of 3D Stem Cell Spheroid

Viability of Neuroblasts under Harsh Conditions



CM: conditioned medium

Therapeutic Potential of 3D Stem Cell Spheroid

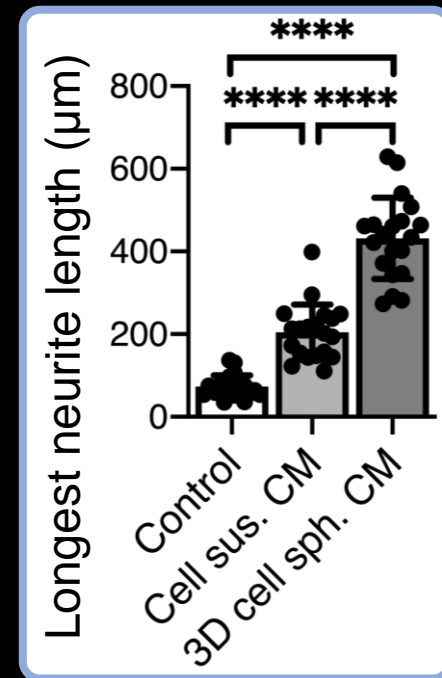
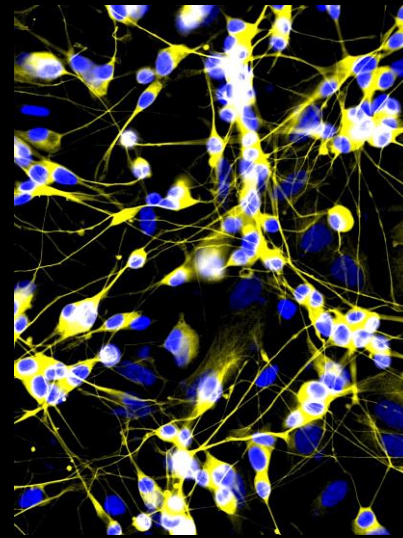
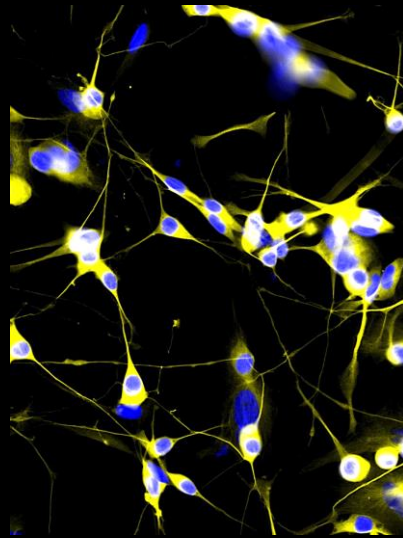
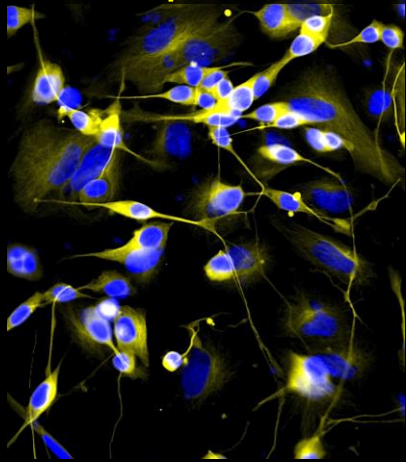
Formation of Neurites by Neuroblasts

Control

Cell sus. CM

3D cell sph. CM

Nuclei β III tubulin

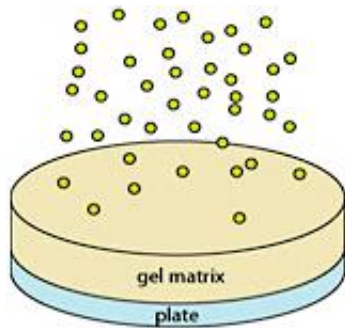


CM: conditioned medium

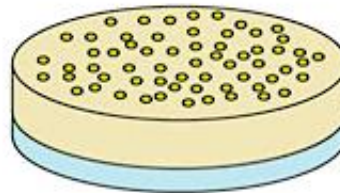
Evaluation of Angiogenic Potential

Tube Formation Assay

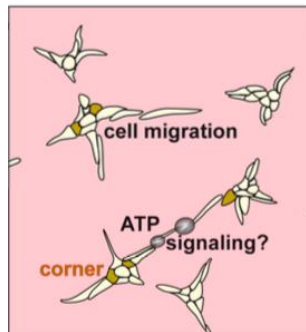
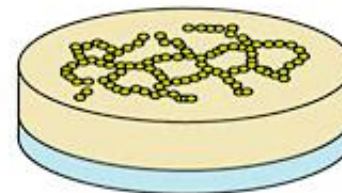
Cell Seeding



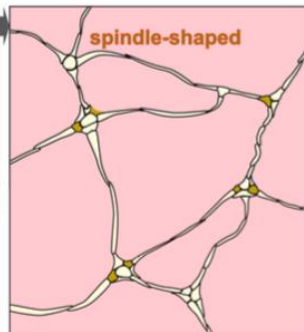
Cell Adhesion



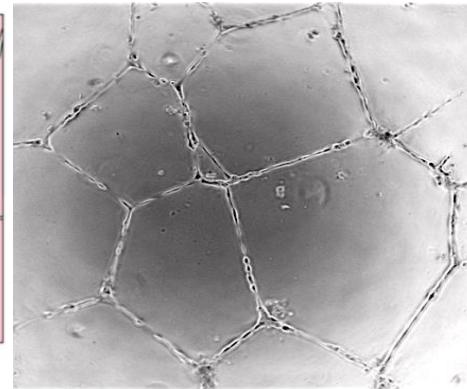
Tube Formation



Cell sprouting

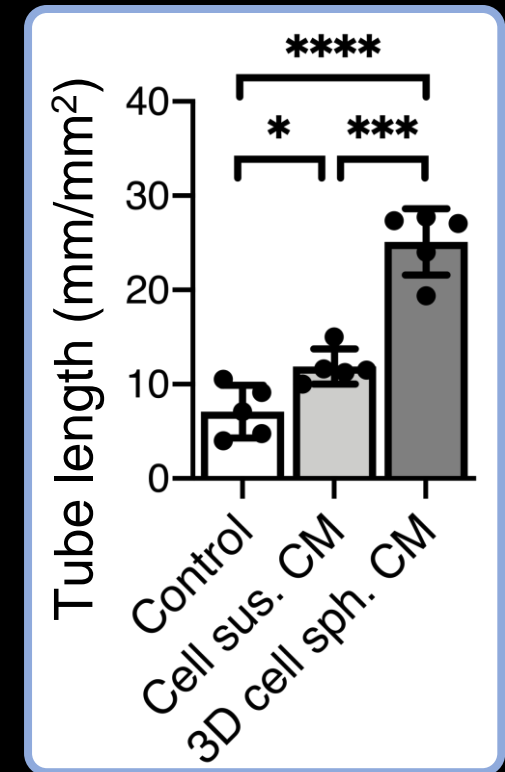
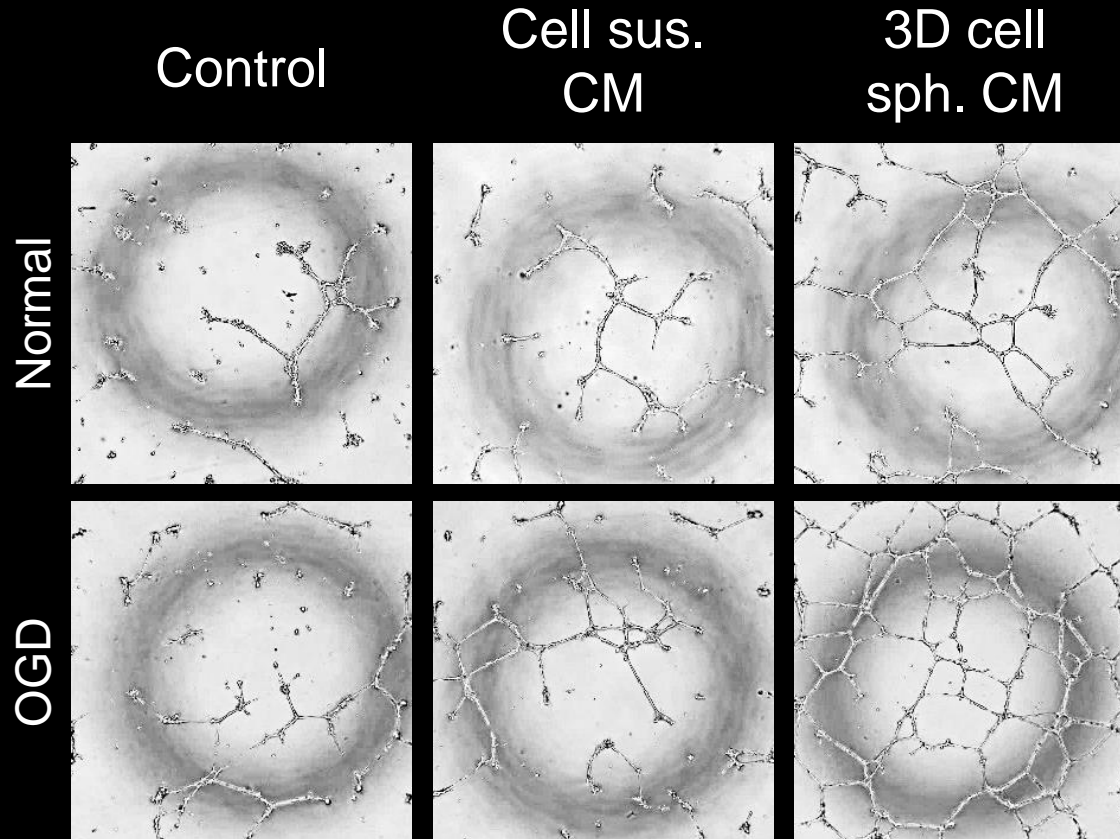


Tube formation



Therapeutic Potential of 3D Stem Cell Spheroid

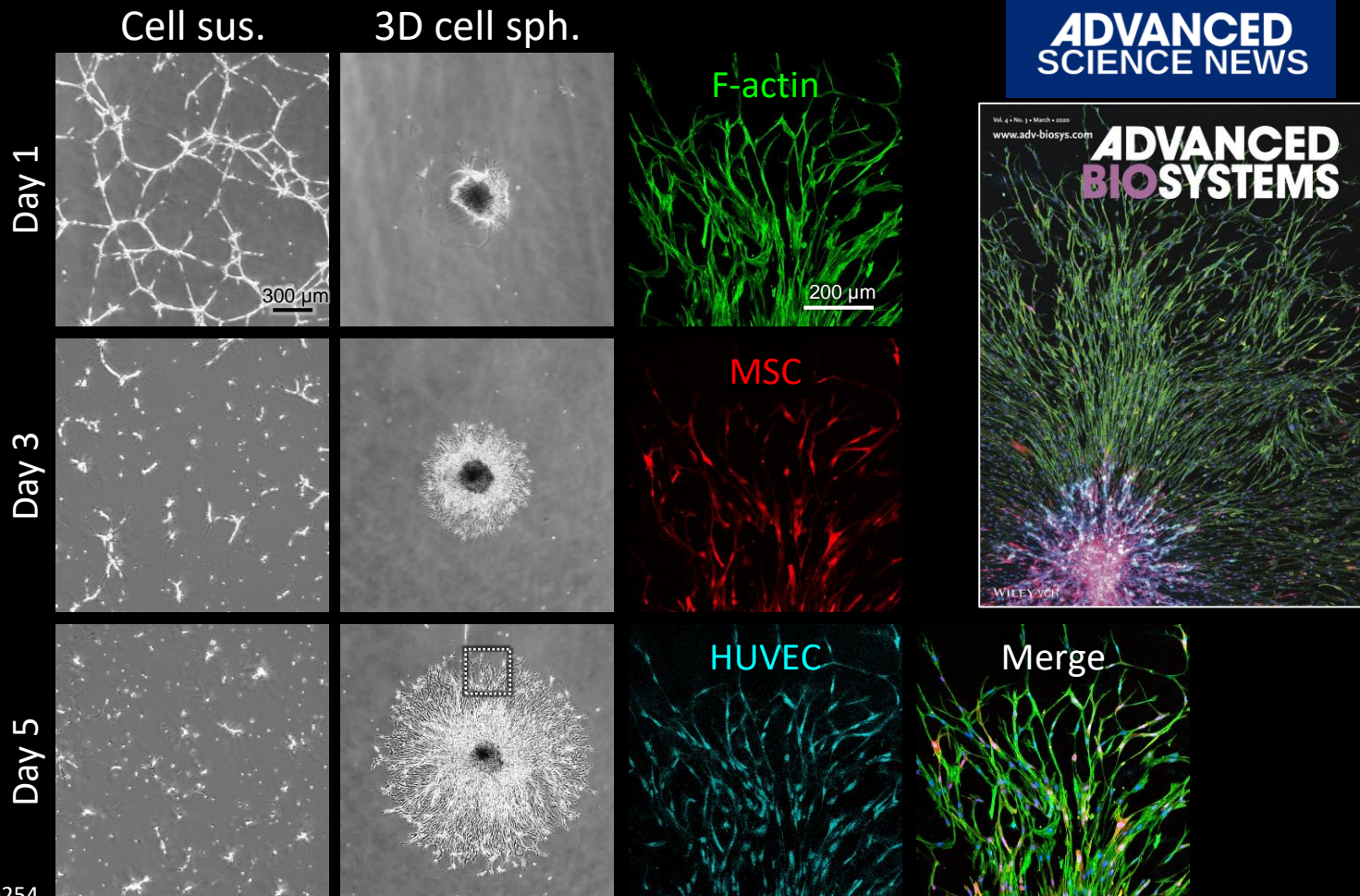
Formation of Tubular Networks by HUVECs



CM: conditioned medium

Therapeutic Potential of 3D Stem Cell Spheroid

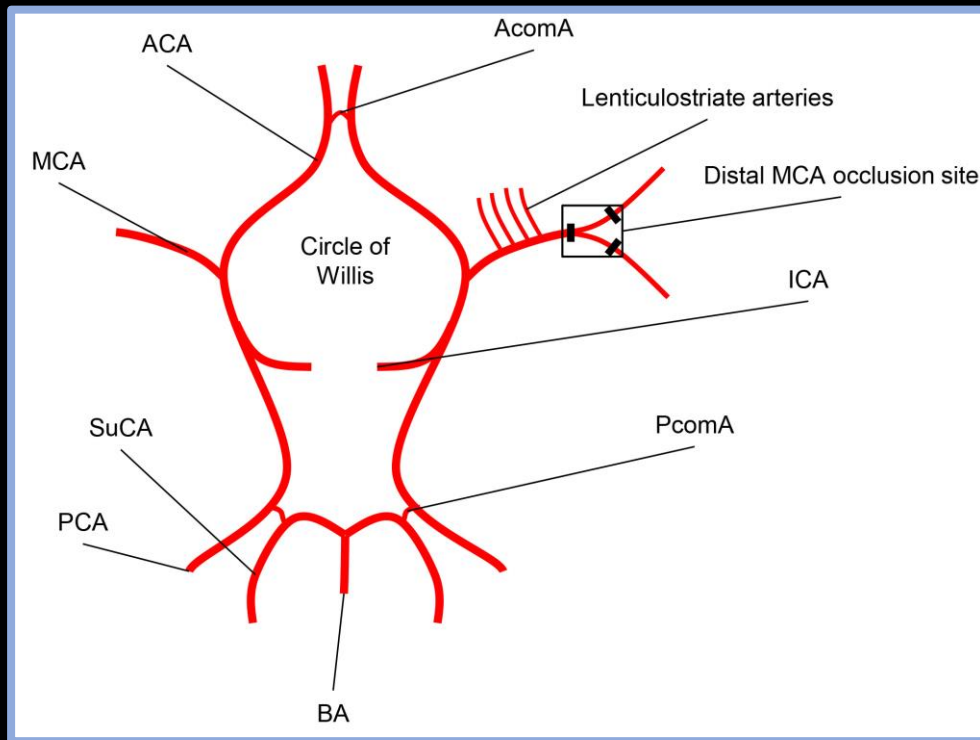
Formation of Tubular Networks by Cell Spheroids



Cell Therapy for Treating Ischemic Stroke

Intracranial Injection

mouse model of middle cerebral artery occlusion



Prof. Yu-Jen Lu
Department of
Neurosurgery, CGMH

Cell Therapy for Treating Ischemic Stroke

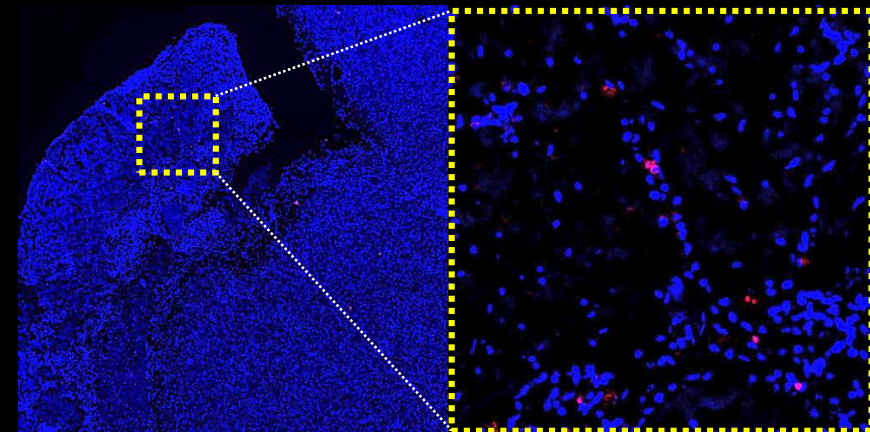
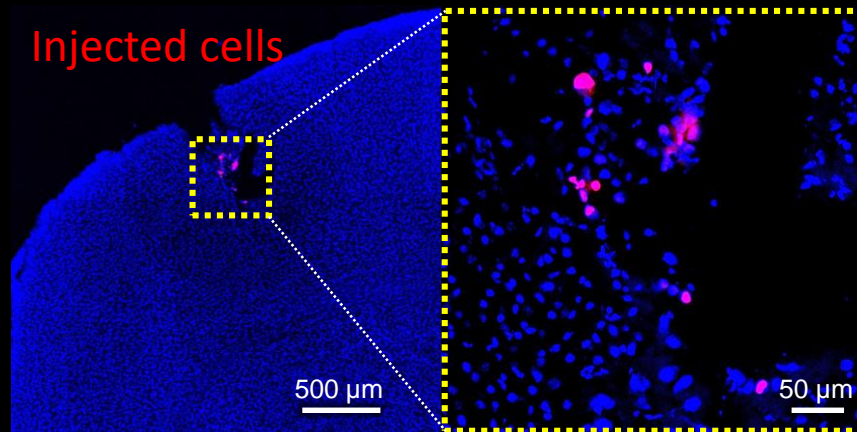
Intracranial Injection

mouse model of middle cerebral artery occlusion

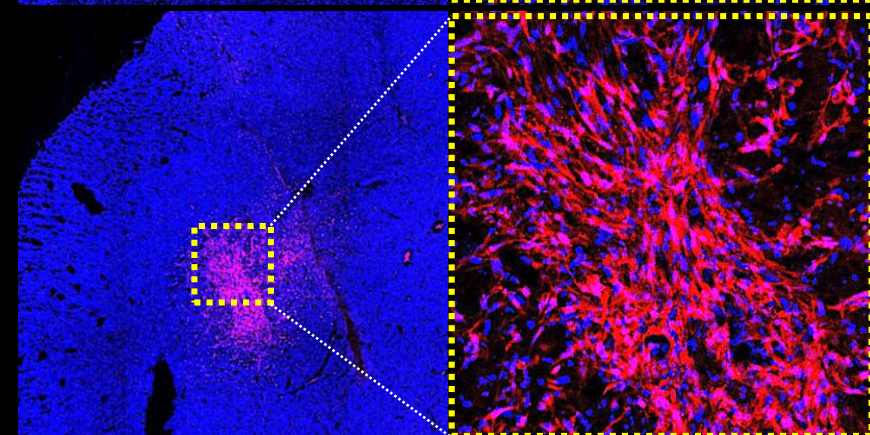
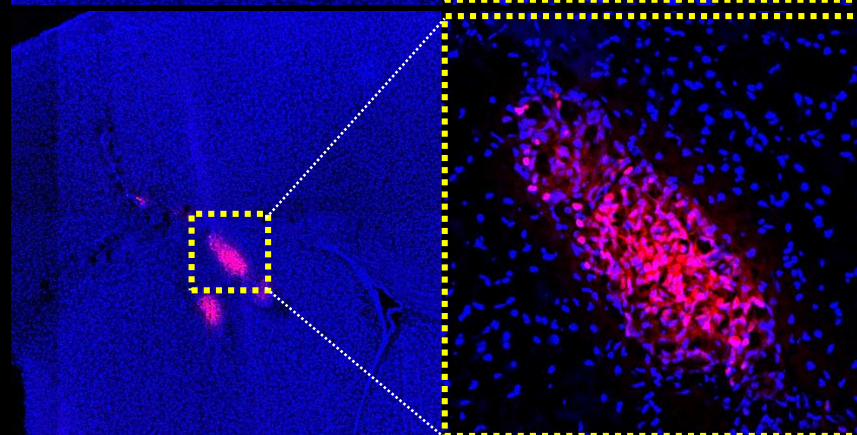
1 h post-op

3 days post-op

Cell suspension

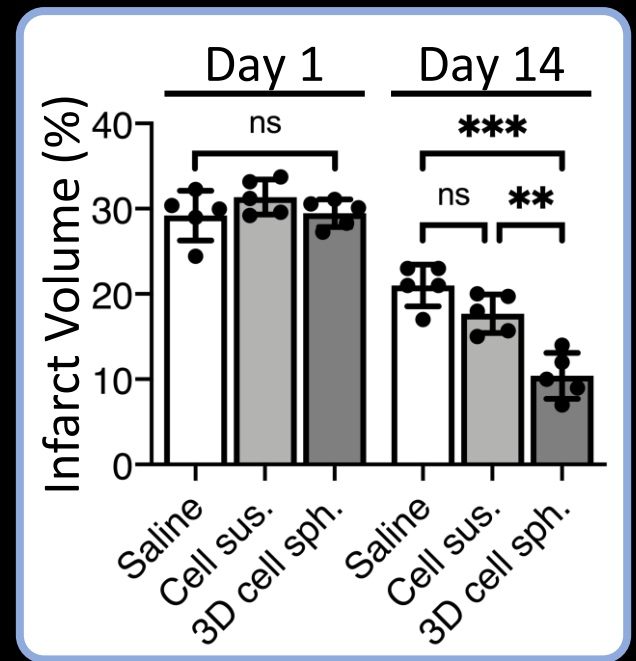
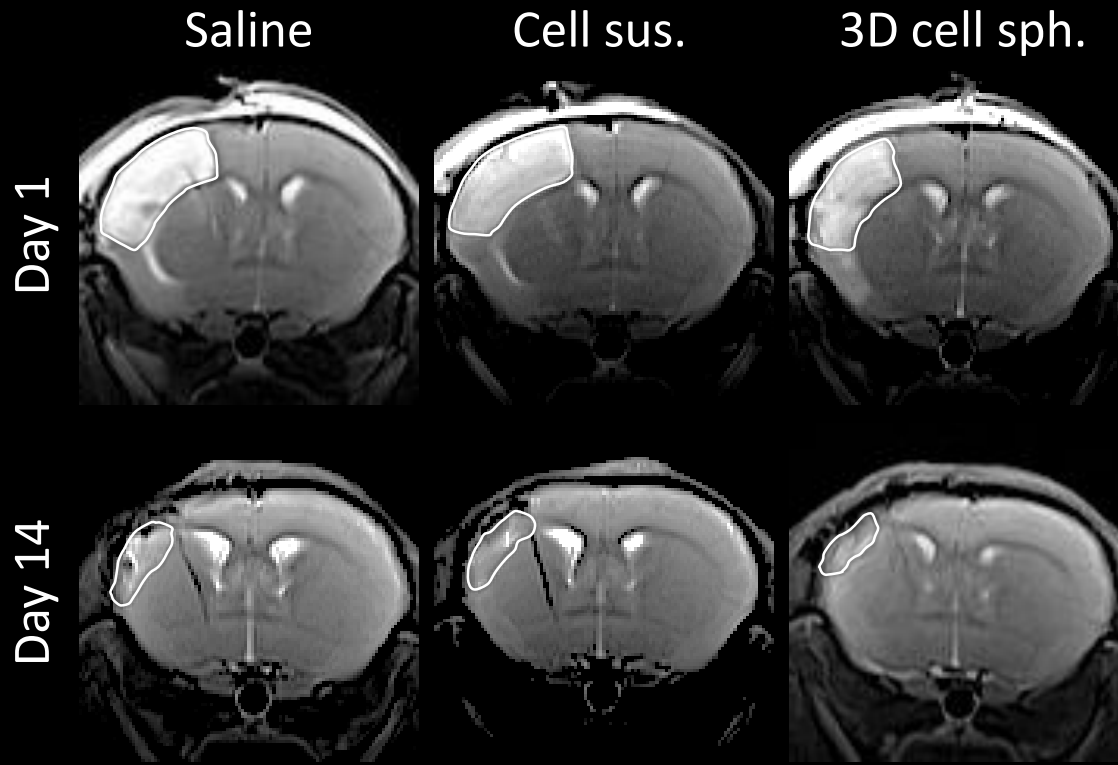


3D cell spheroid



Cell Therapy for Treating Ischemic Stroke

Magnetic Resonance Imaging



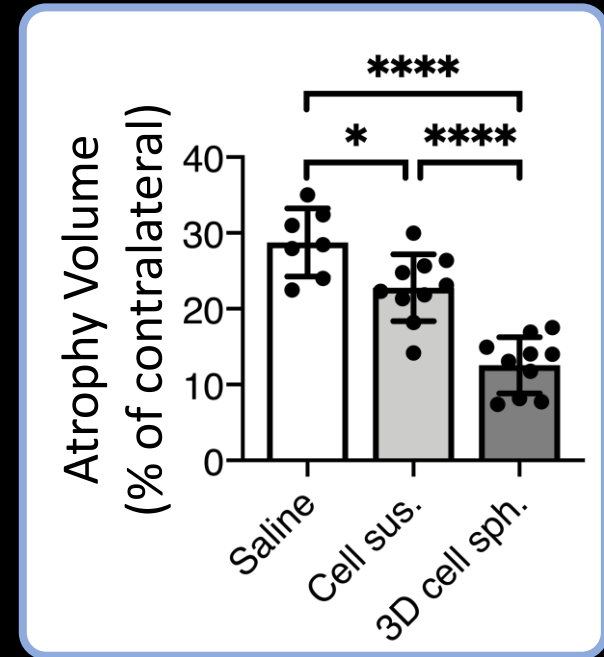
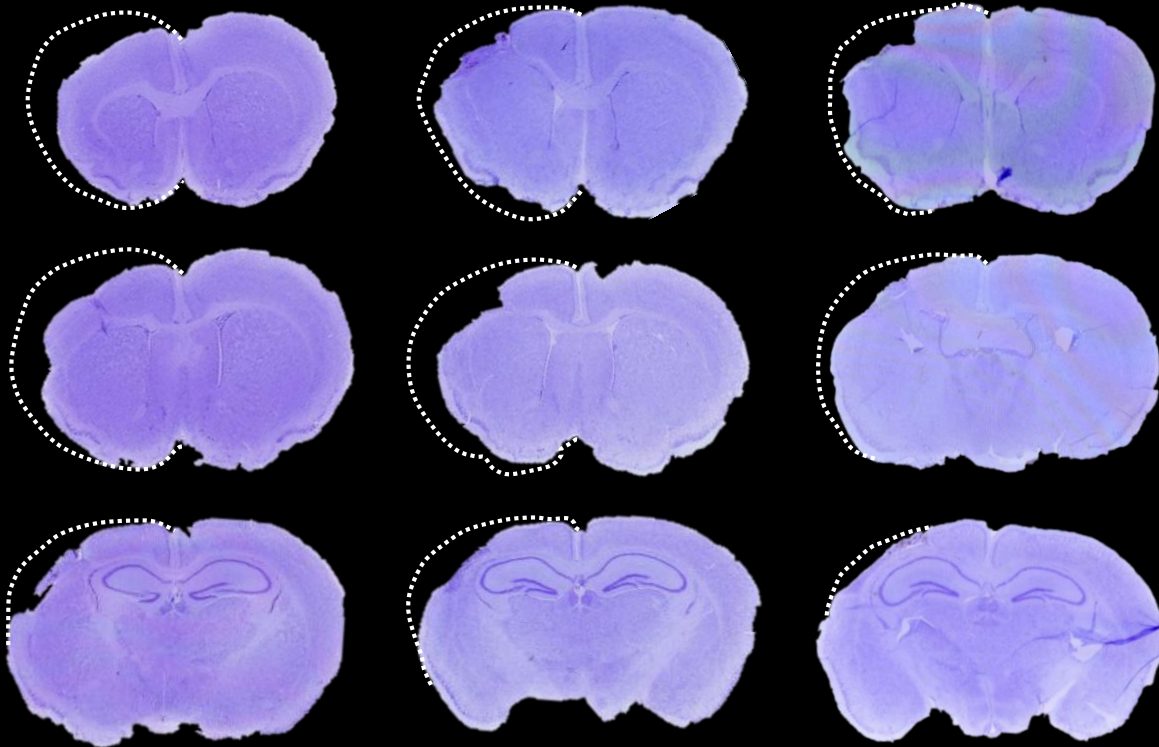
Cell Therapy for Treating Ischemic Stroke

Cresyl Violet Staining

Saline

Cell sus.

3D cell sph.



Cell Therapy for Treating Ischemic Stroke

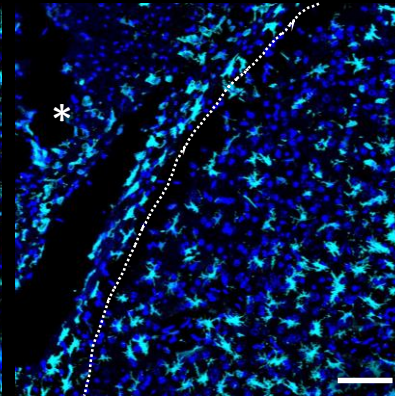
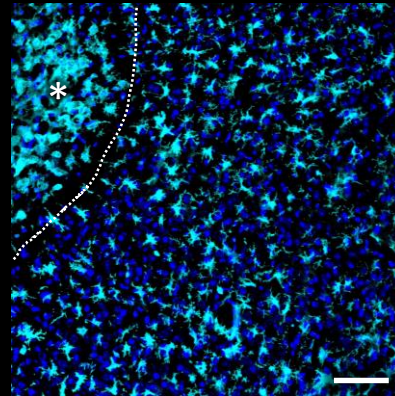
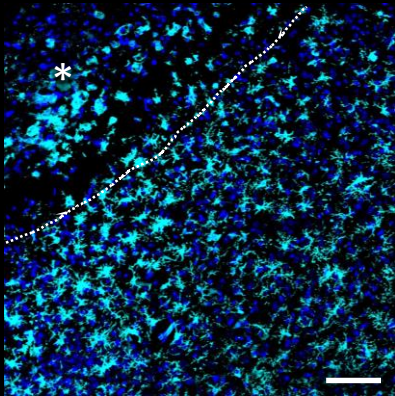
Microglial Cells & Astrocytes

Saline

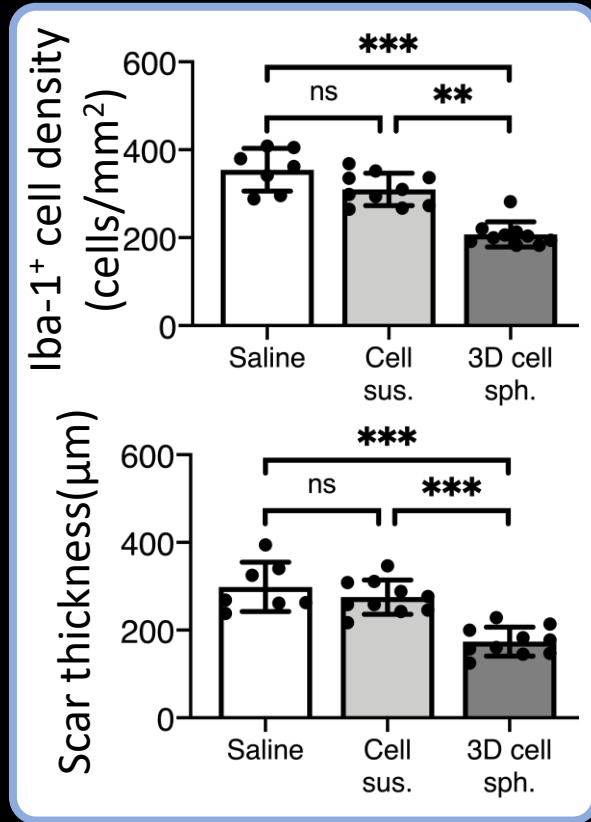
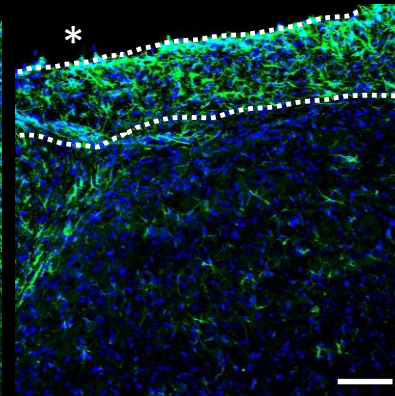
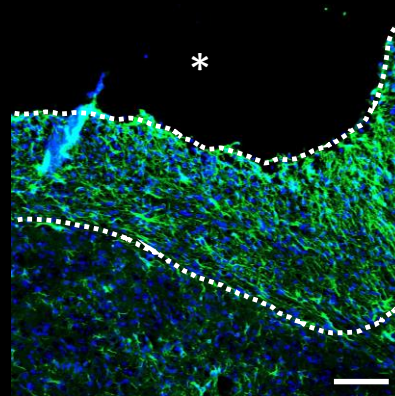
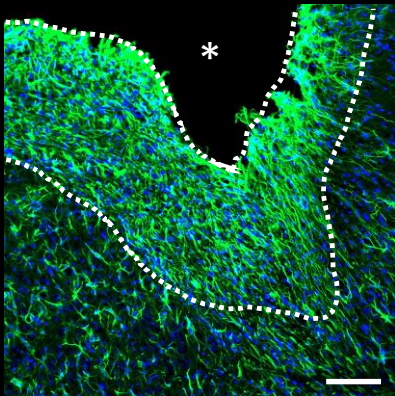
Cell sus.

3D cell sph.

Nuclei Iba-1



Nuclei GFAP



Cell Therapy for Treating Ischemic Stroke

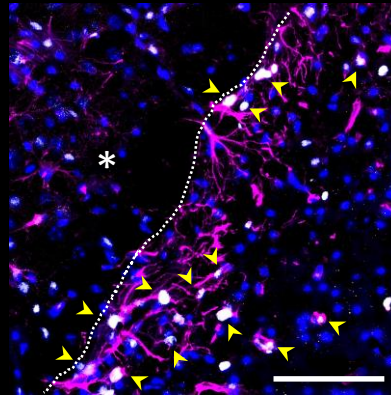
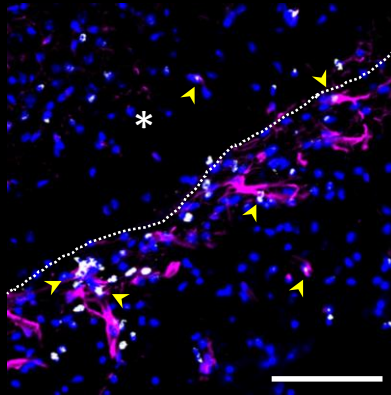
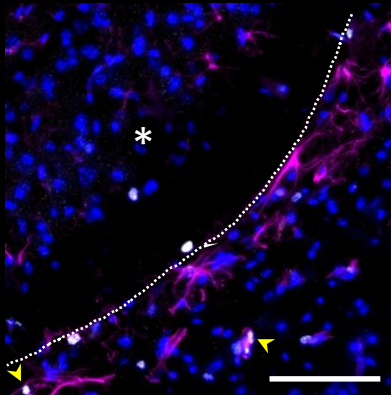
Neurogenesis & Angiogenesis

Saline

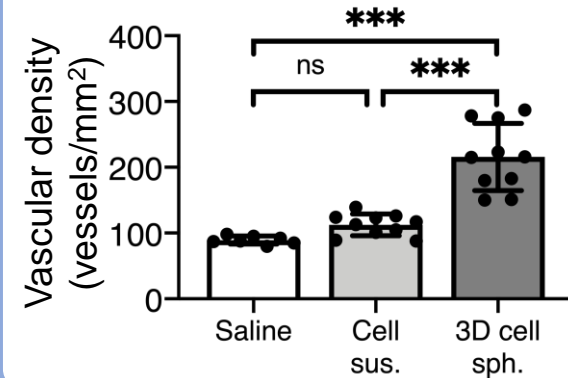
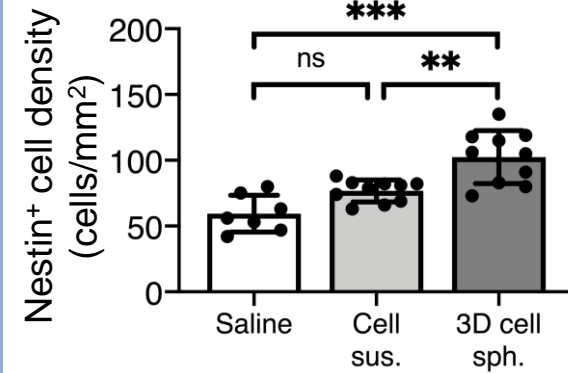
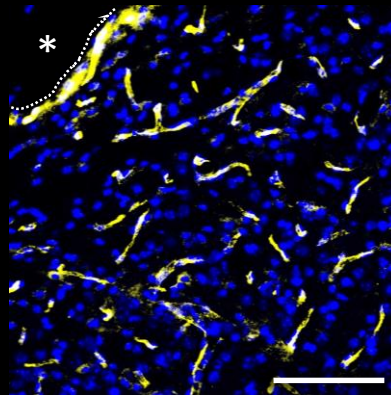
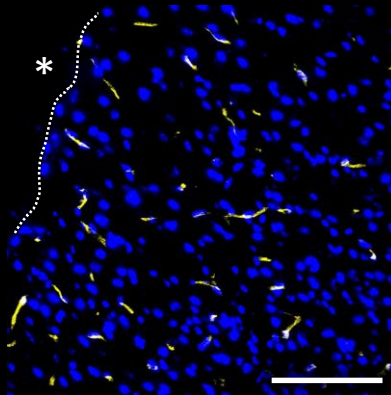
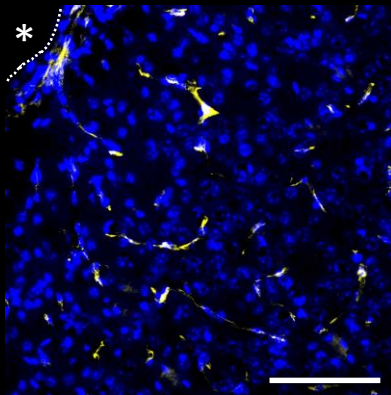
Cell sus.

3D cell sph.

Nuclei Nestin Ki67



Nuclei CD31



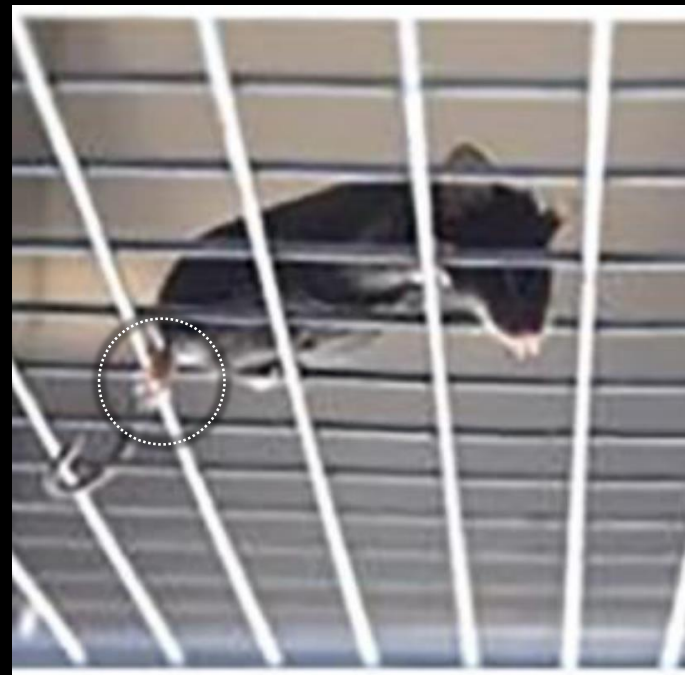
Cell Therapy for Treating Ischemic Stroke

Motor Functional Recovery

Cylinder test:
forelimb asymmetry



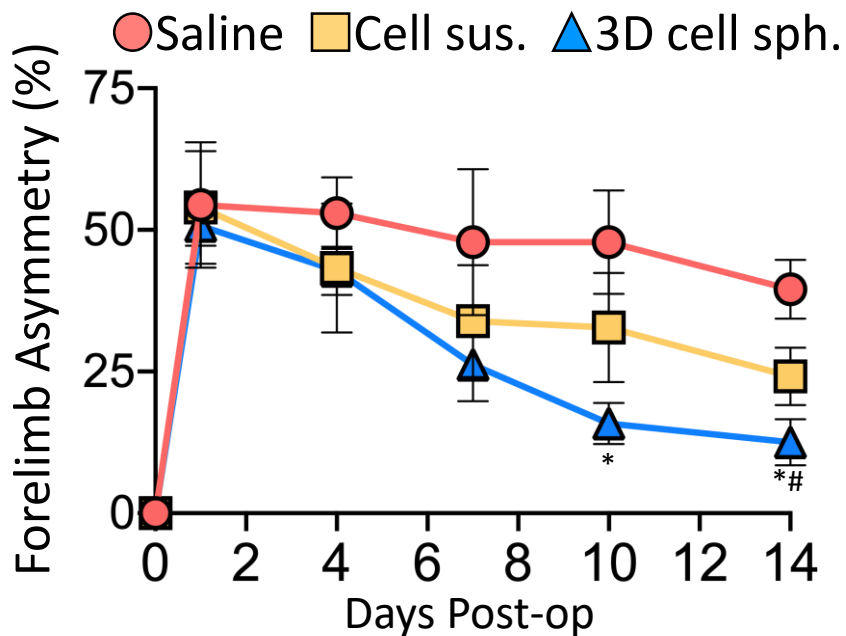
Grid test:
hindlimb foot fault



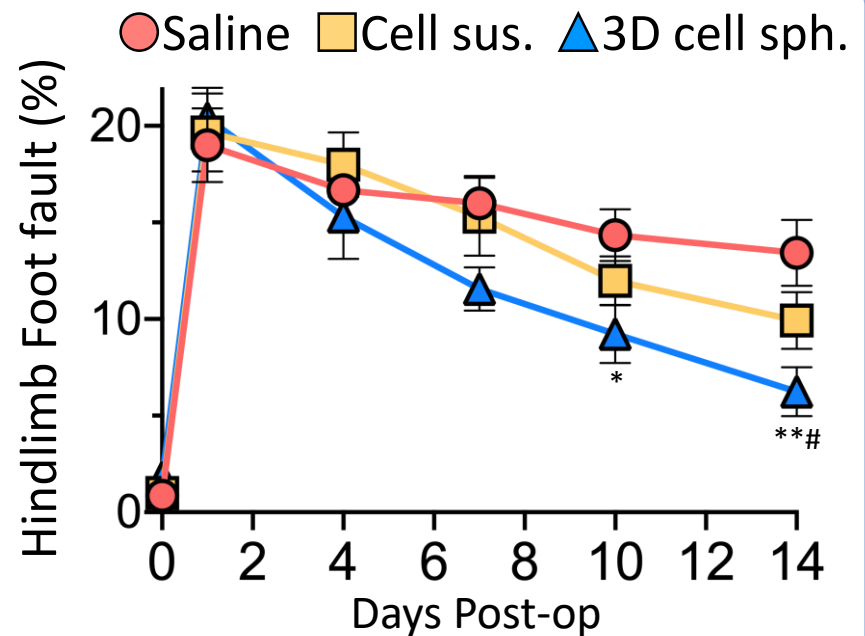
Cell Therapy for Treating Ischemic Stroke

Motor Functional Recovery

Cylinder test:
forelimb asymmetry



Grid test:
hindlimb foot fault



Summary (I)

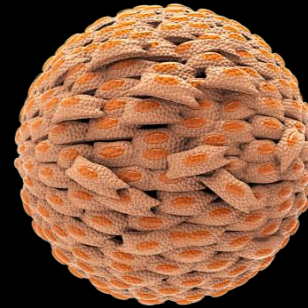
Single cell suspension



Cell only

vs.

3D cell spheroid



Cell + ECM +
soluble factors

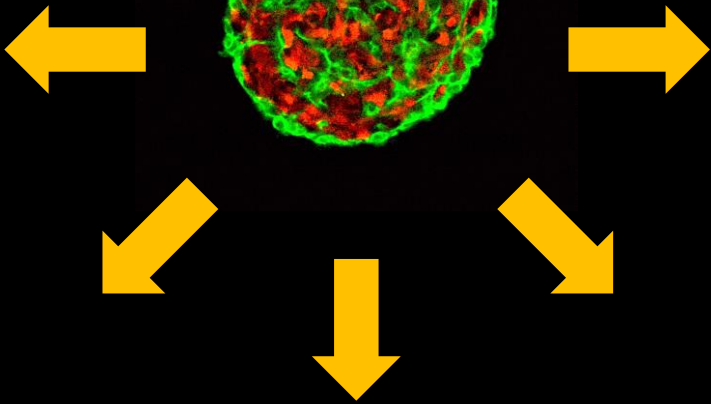
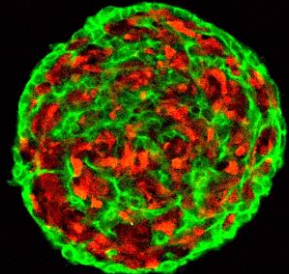
3D cell spheroids:

- Maintain cell viability
- Enhance cell delivery efficiently
- **Increase therapeutic efficacy**

Cell Therapy Using 3D Stem Cell Spheroids

Ischemic stroke
Traumatic brain injury

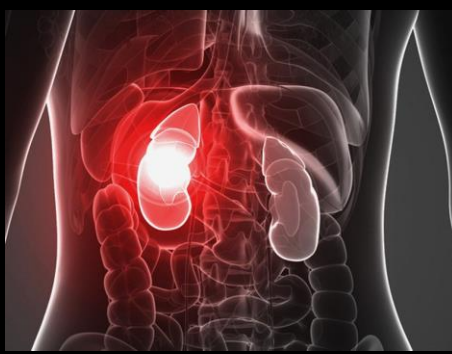
Type I
diabetes mellitus



Peripheral nerve injury

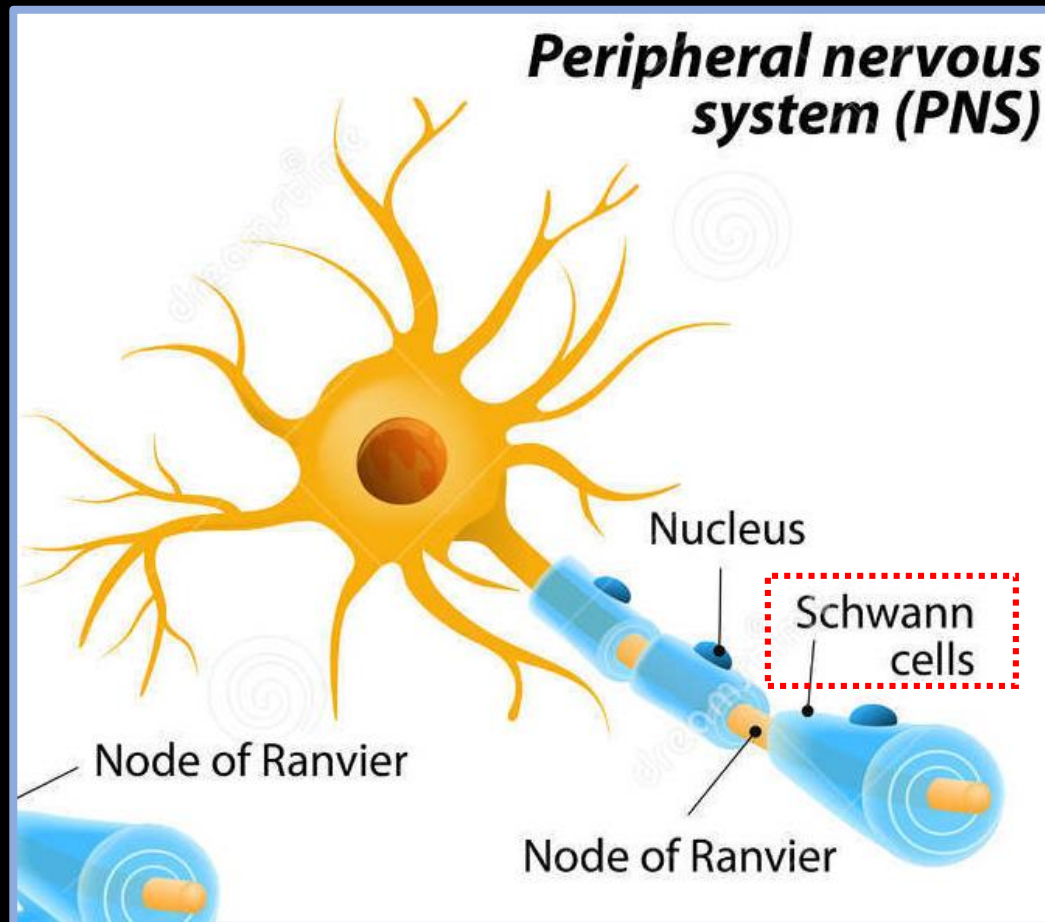
Kidney injury

Tendon/ligament
injury



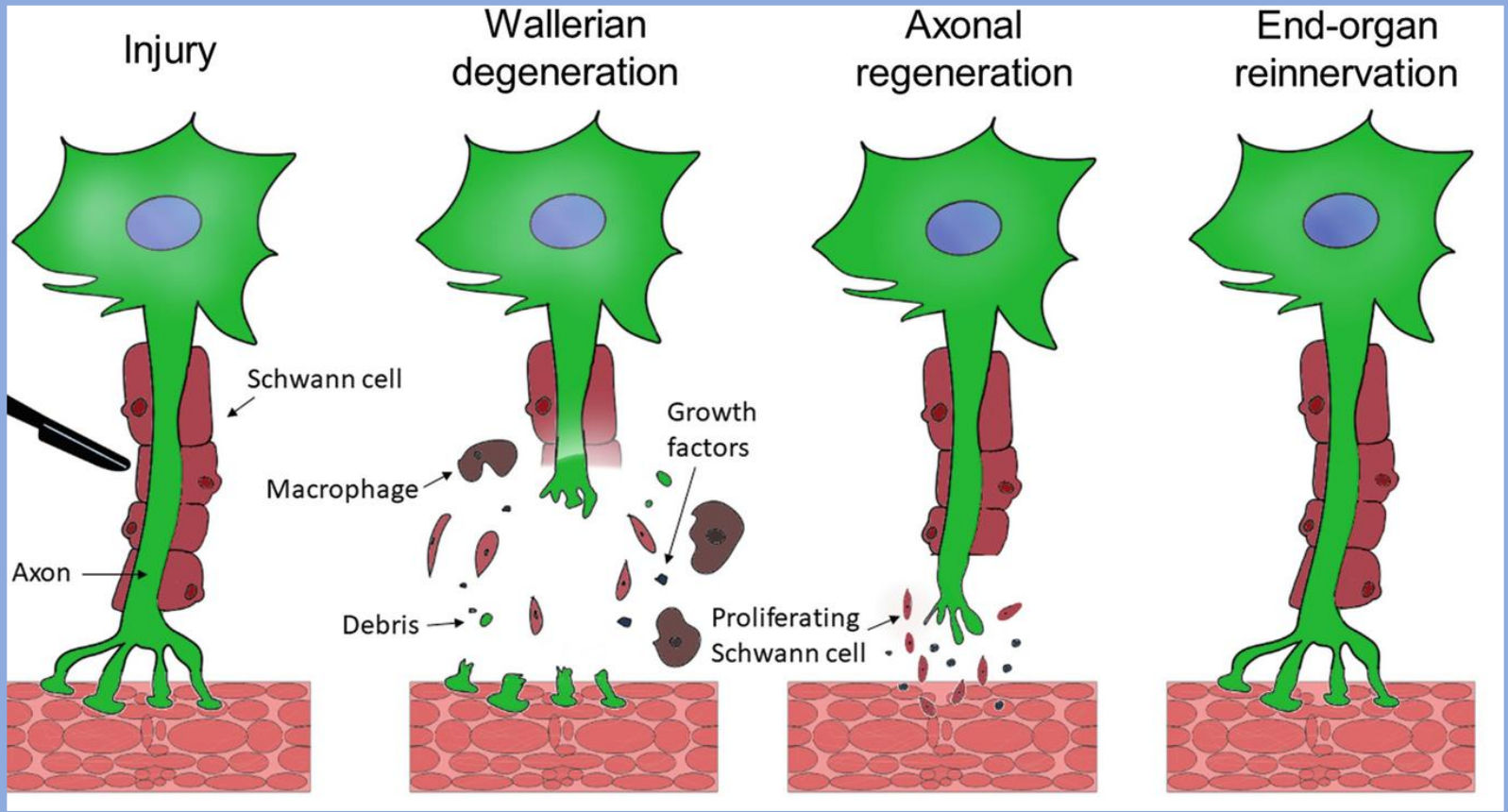
Cell Therapy for Peripheral Nerve Injury

Schwann Cells & Nerve Repair



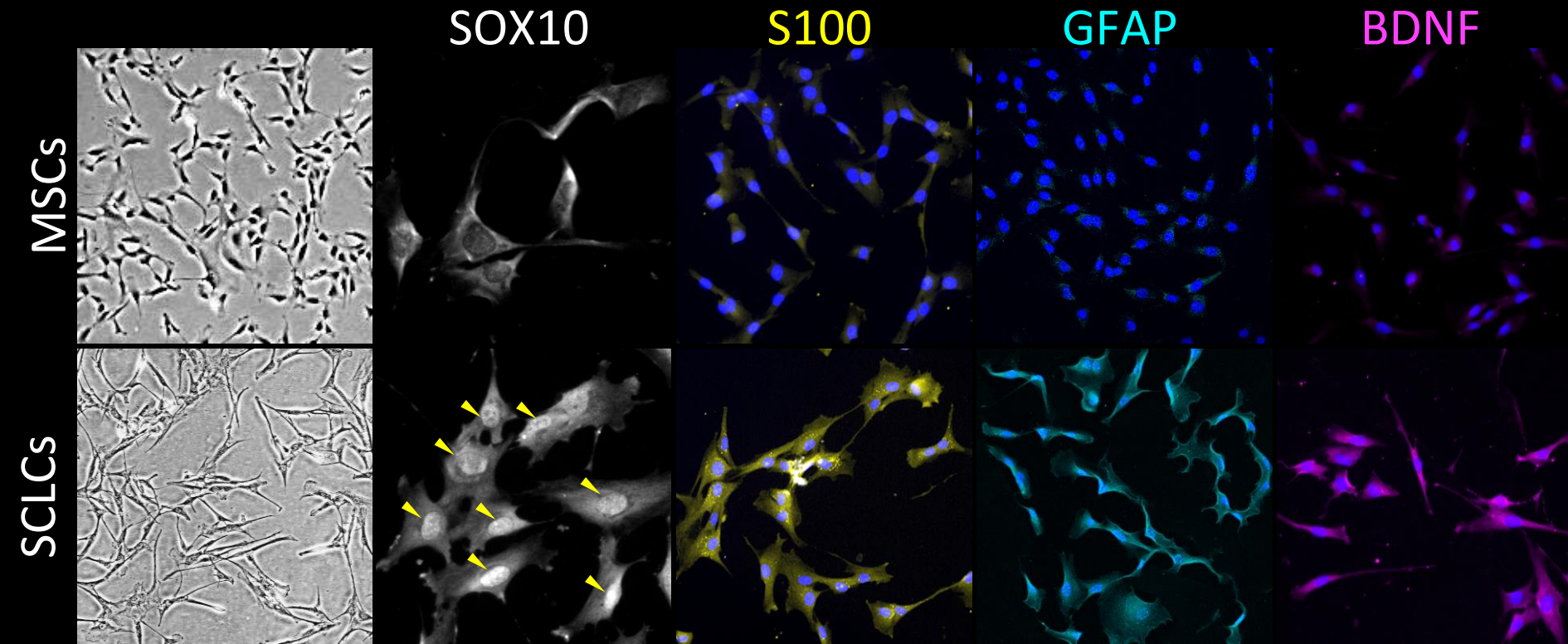
Cell Therapy for Peripheral Nerve Injury

Schwann Cells & Nerve Repair



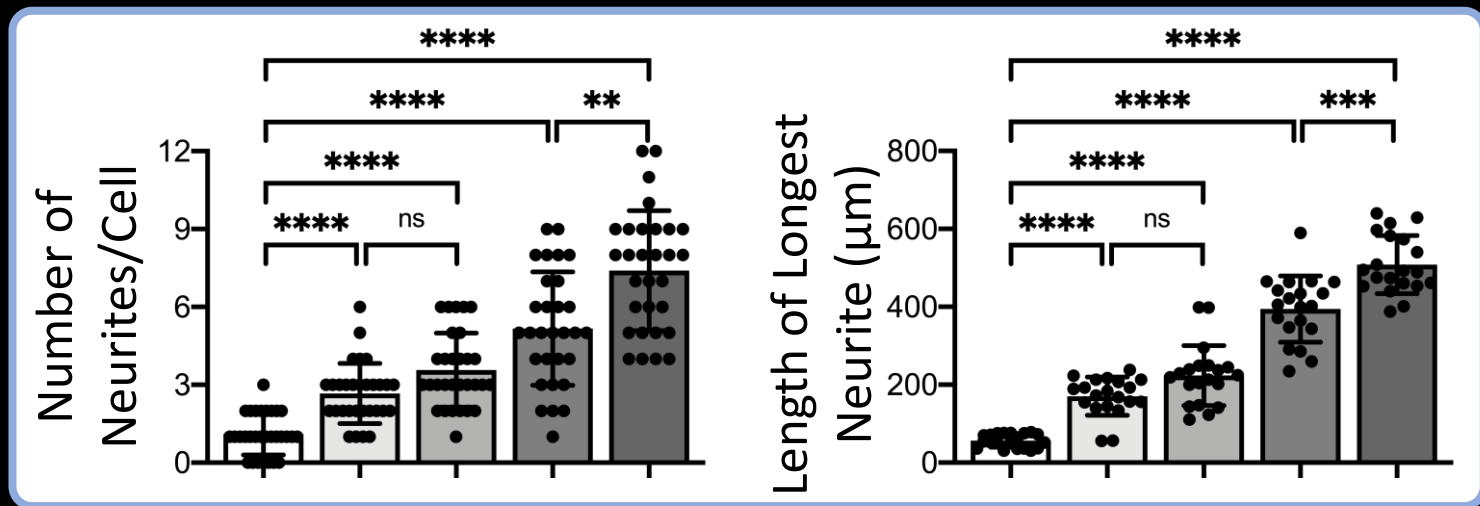
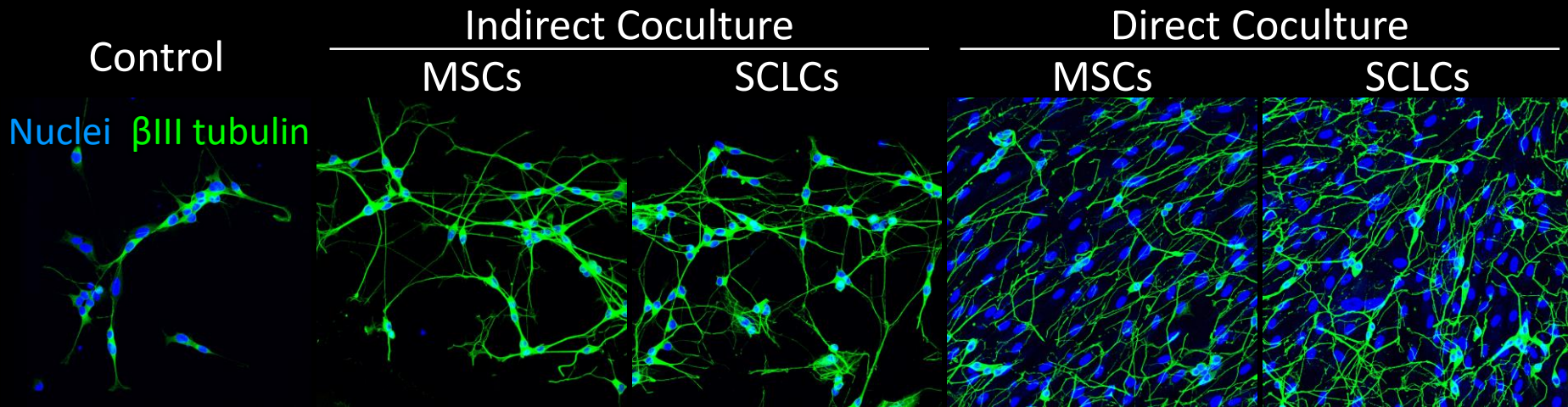
Cell Therapy for Peripheral Nerve Injury

MSC-derived Schwann Cell-like Cells (SCLCs)



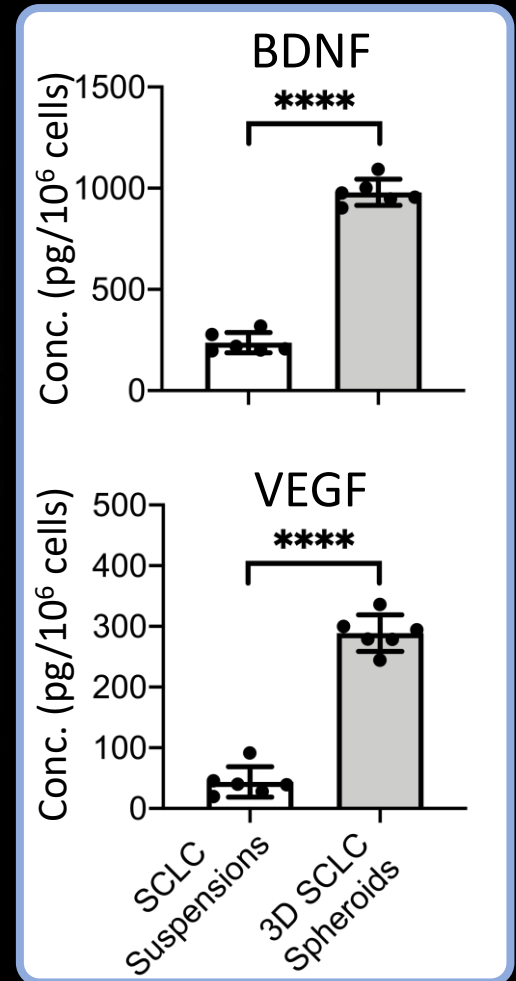
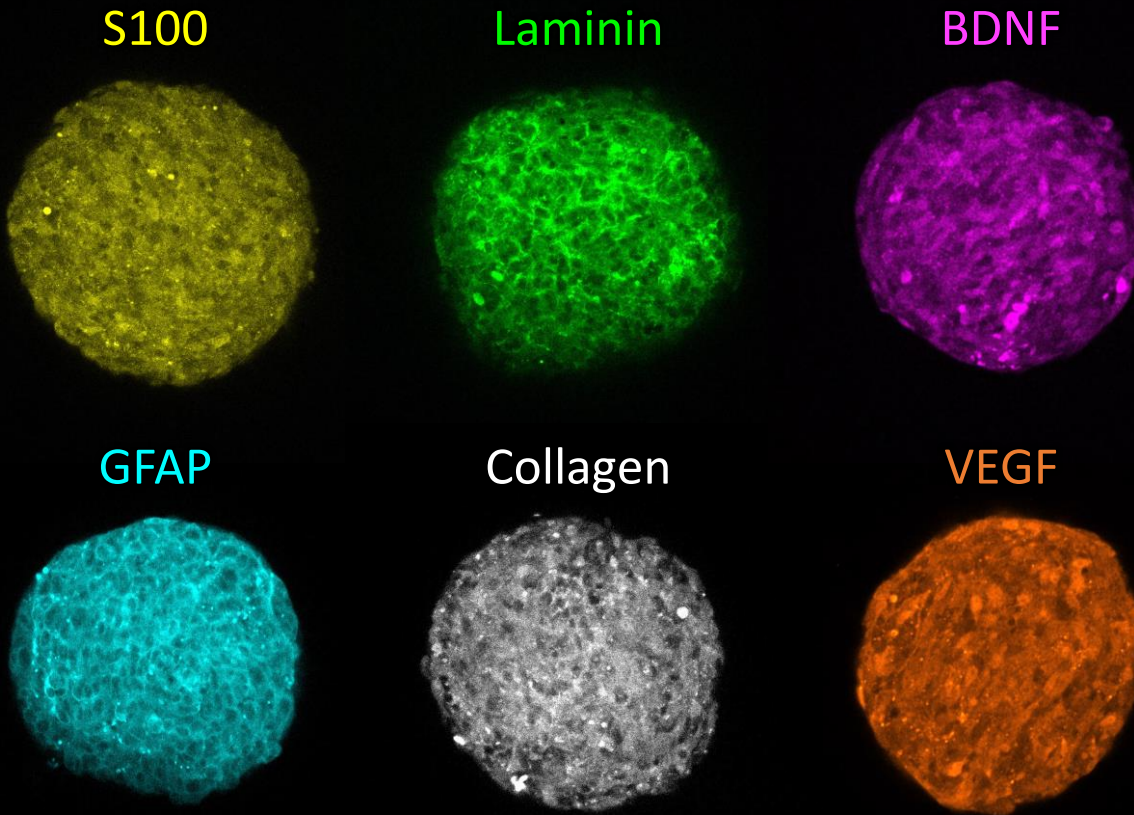
Cell Therapy for Peripheral Nerve Injury

MSC-derived SCLCs Promote Neurite Formation



Cell Therapy for Peripheral Nerve Injury

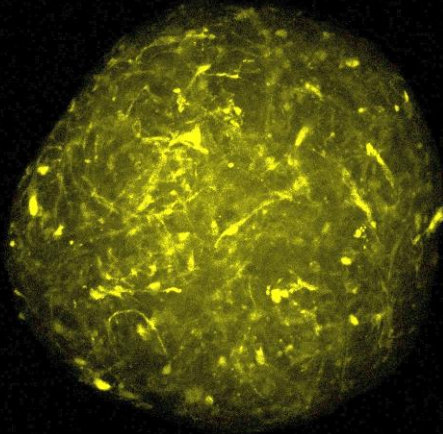
3D Spheroids of MSC-derived SCLCs



Cell Therapy for Peripheral Nerve Injury

3D Spheroids of Primary Schwann Cells

S100

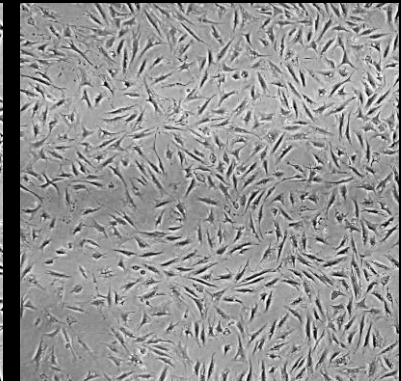
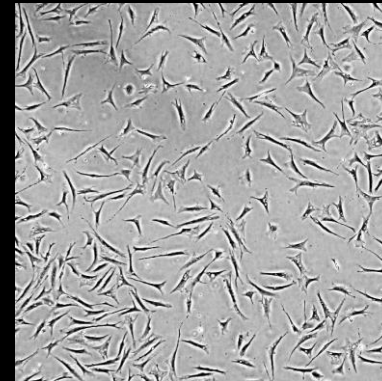
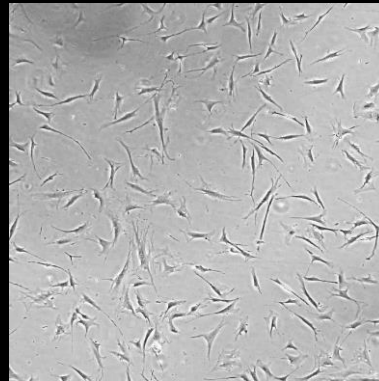


Primary Schwann cells

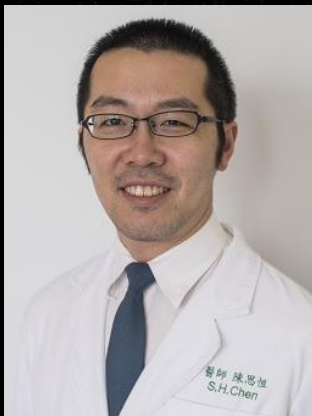
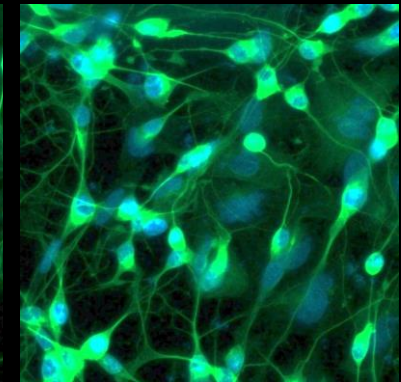
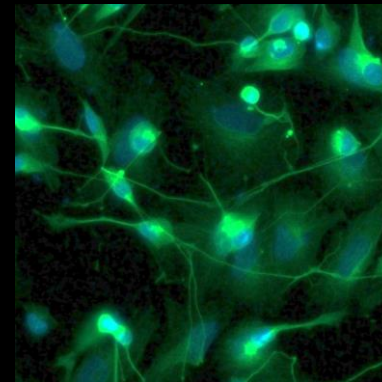
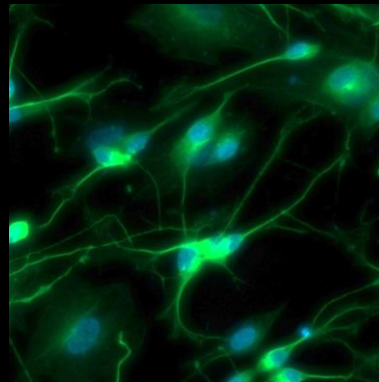
Control

Cell sus. CM

3D cell sph. CM



Neuroblasts



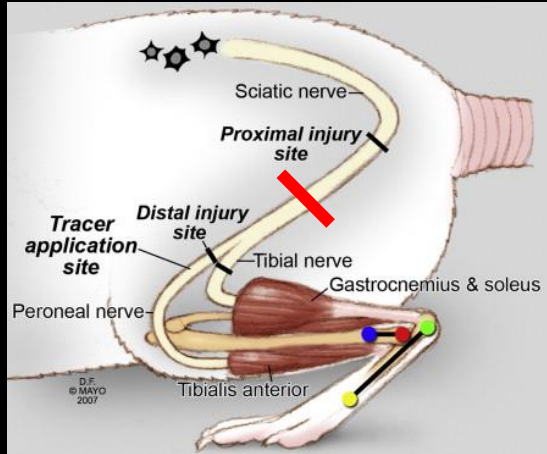
Prof. Shih-Heng Chen

Division of Plastic Surgery, CGMH

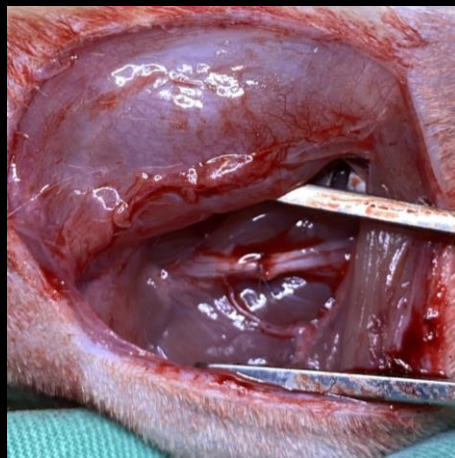
Cell Therapy for Peripheral Nerve Injury

Transplantation of 3D Cell Spheroids into Sciatic Nerve

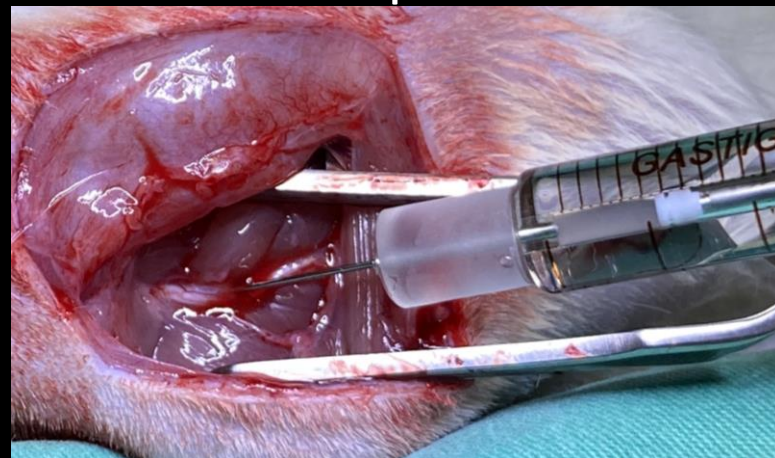
Transection



Suture

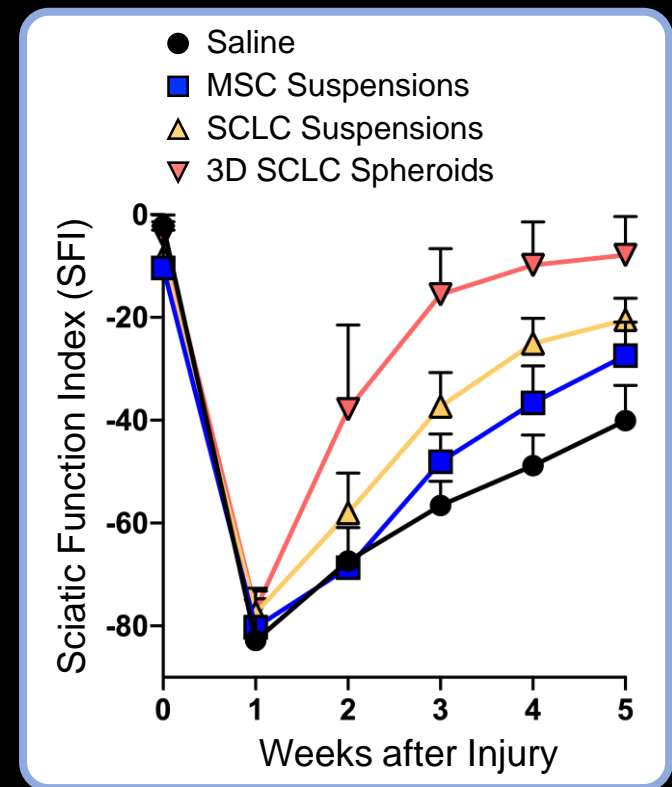
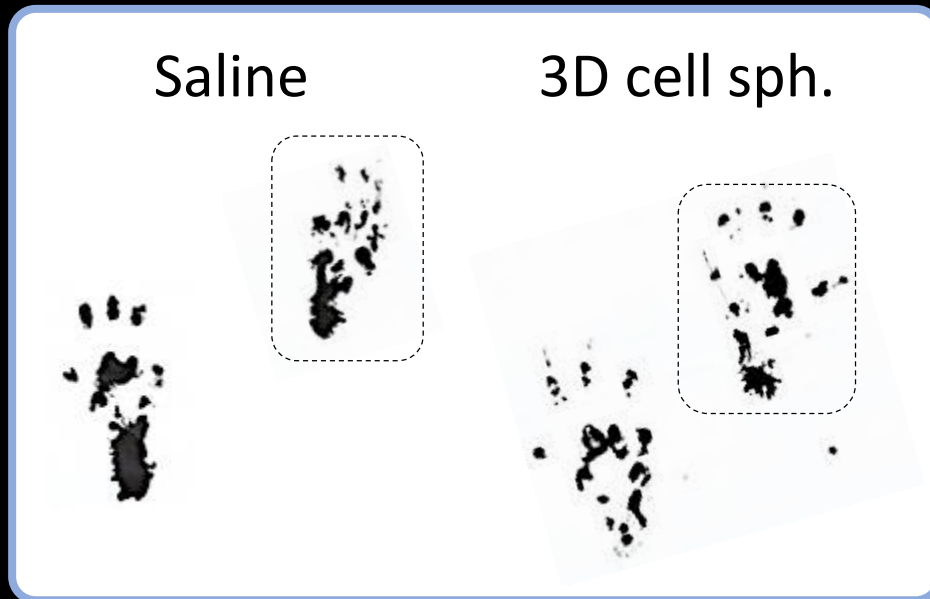


Cell Transplantation



Cell Therapy for Peripheral Nerve Injury

Gait Analysis



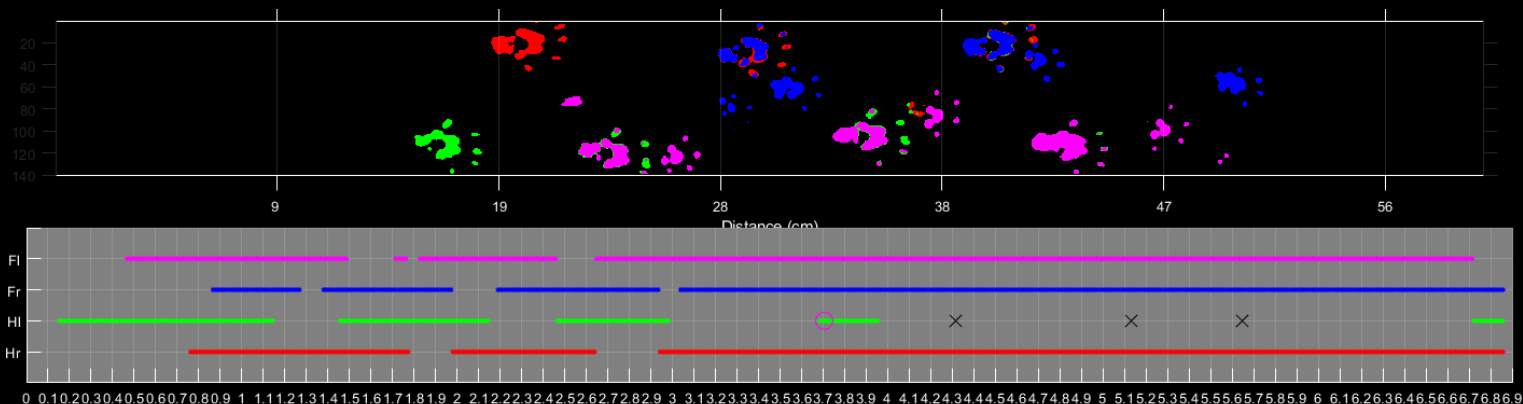
Cell Therapy for Peripheral Nerve Injury

Gait Analysis



Prof. Er-Yuan Chuang
Graduate Institute of
Biomedical Materials and
Tissue Engineering, TMU

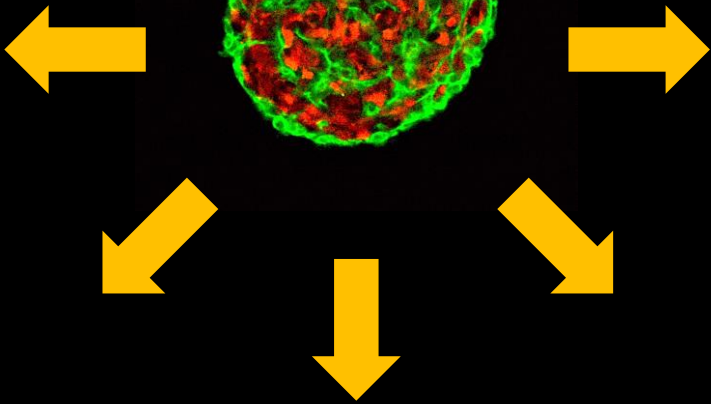
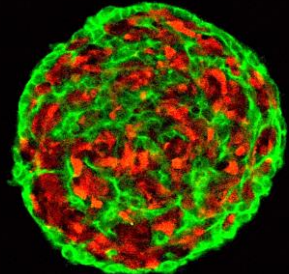
GaitLab in TMU Animal Center



Cell Therapy Using 3D Stem Cell Spheroids

Ischemic stroke
Traumatic brain injury

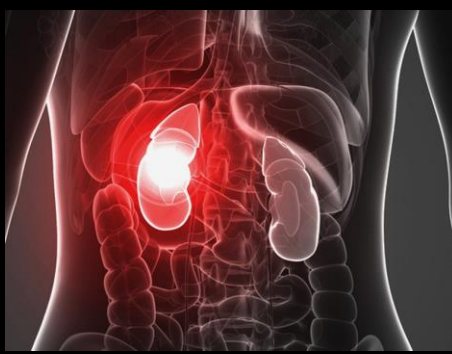
Type I
diabetes mellitus



Peripheral nerve injury

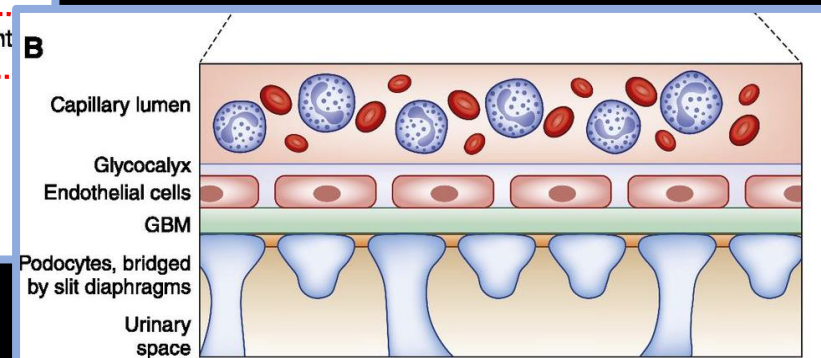
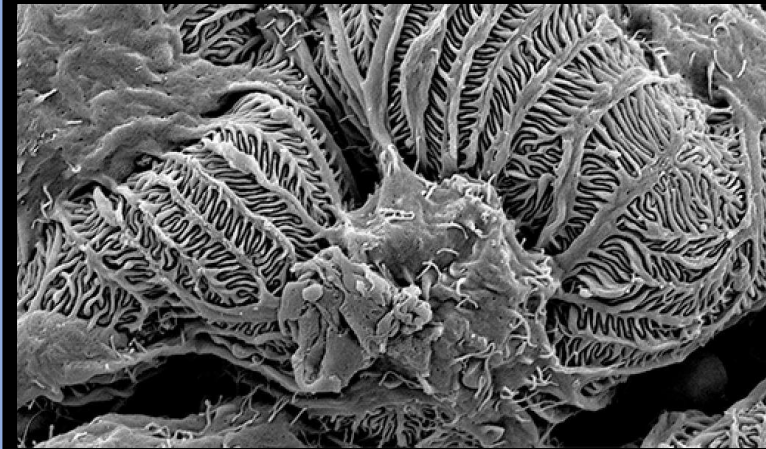
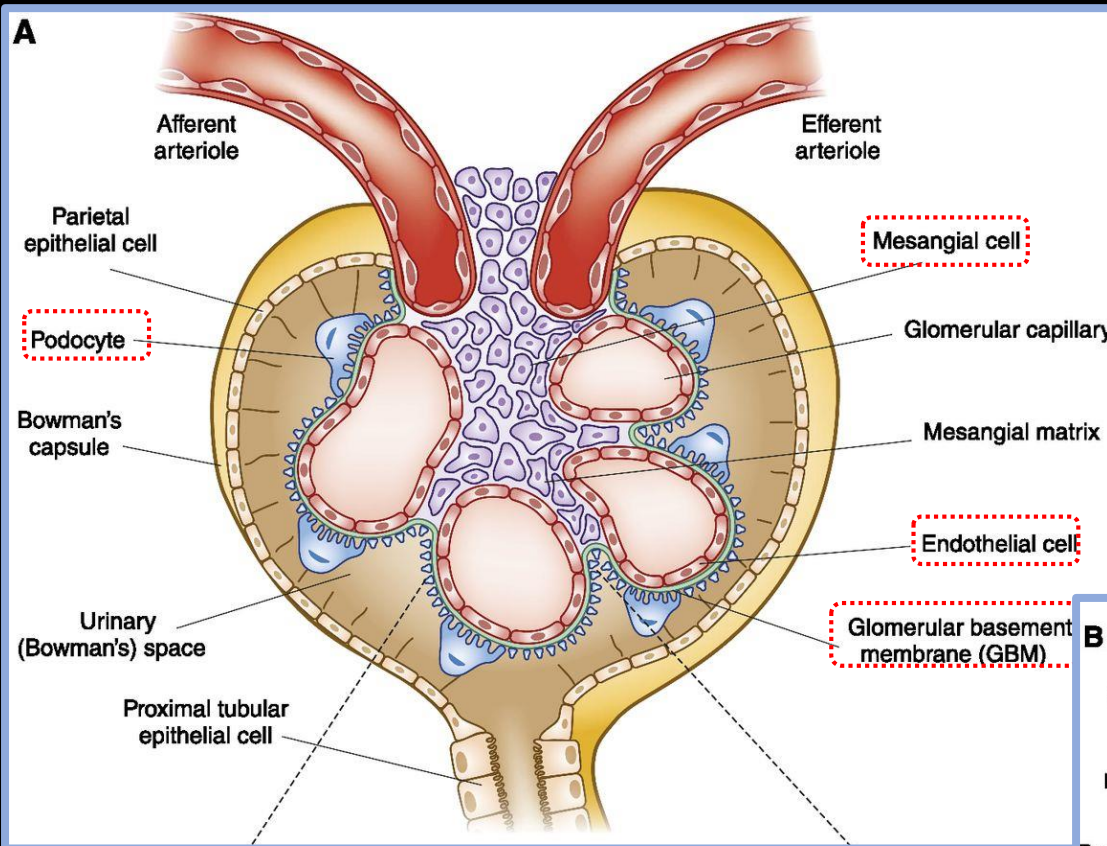
Kidney injury

Tendon/ligament
injury



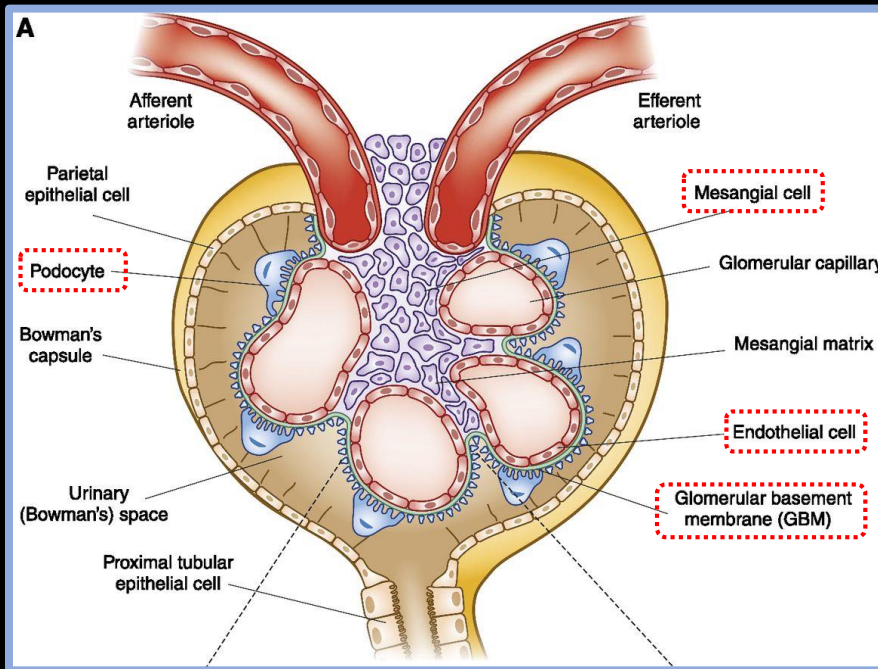
Cell Therapy for Kidney Injury

Engineer a Glomerulus-like Microenvironment



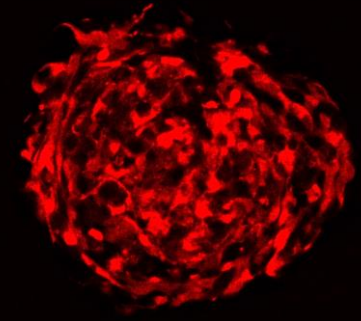
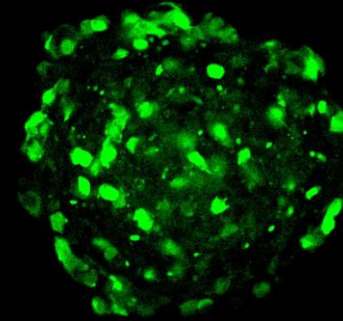
Cell Therapy for Kidney Injury

Engineer a Glomerulus-like Microenvironment



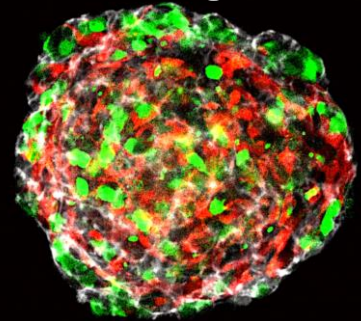
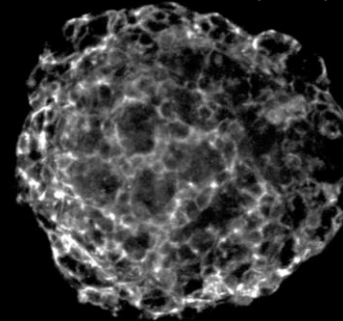
Podocyte; P (ms)

MSC; M (hu)



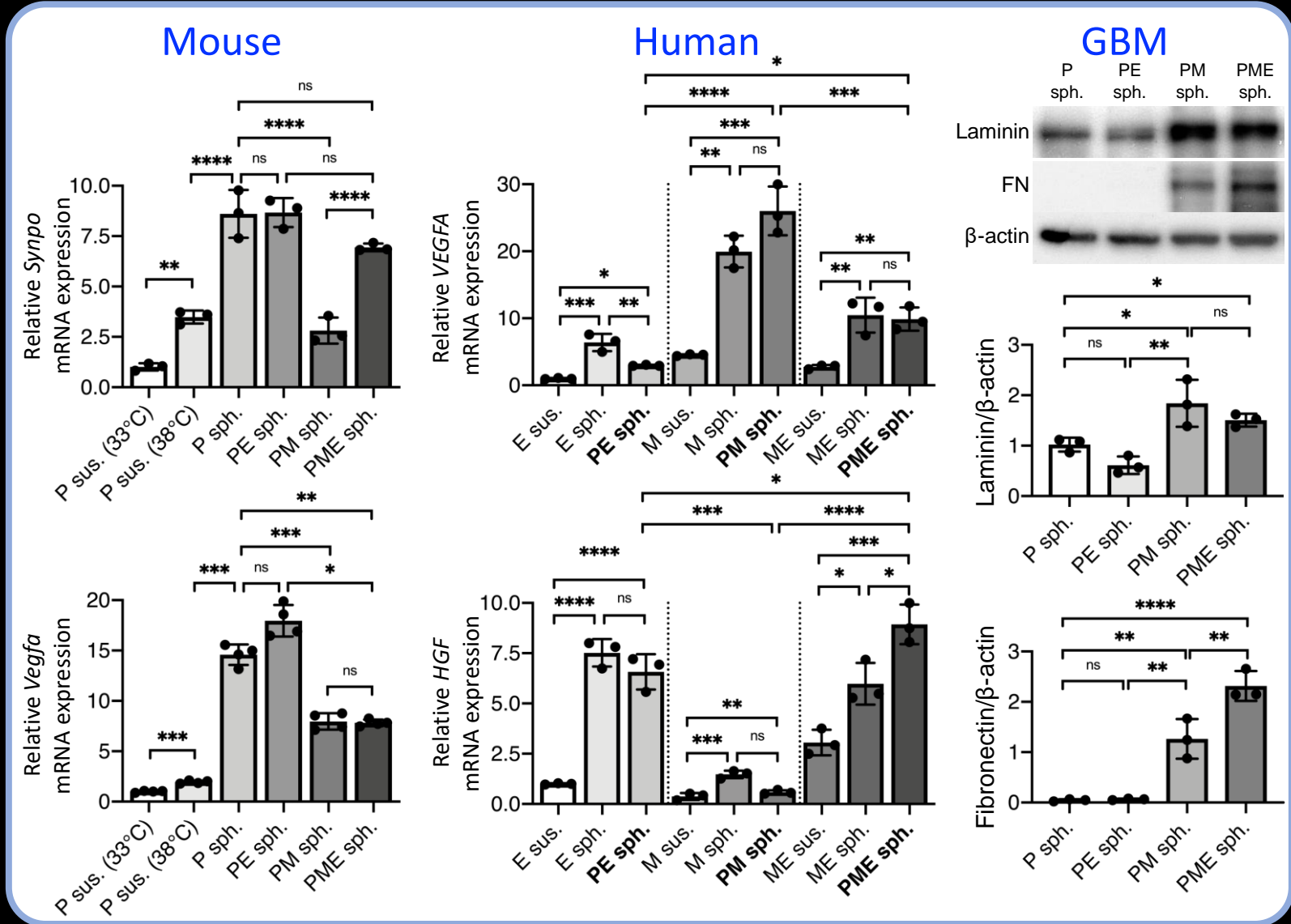
HUVEC; E (hu)

Merge



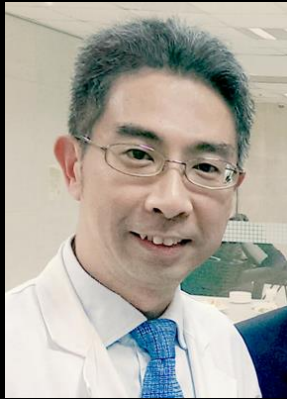
Cell Therapy for Kidney Injury

Engineer a Glomerulus-like Microenvironment

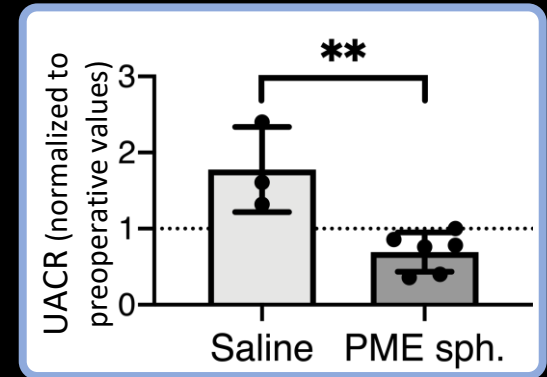
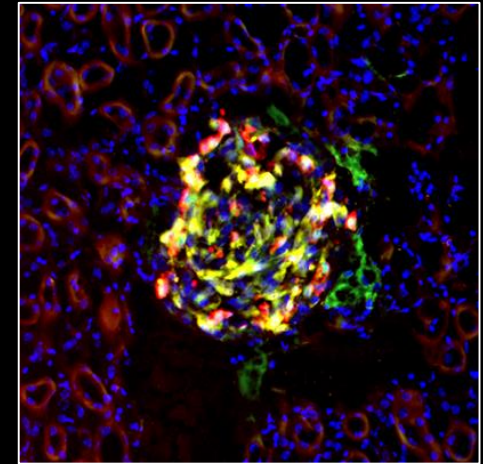
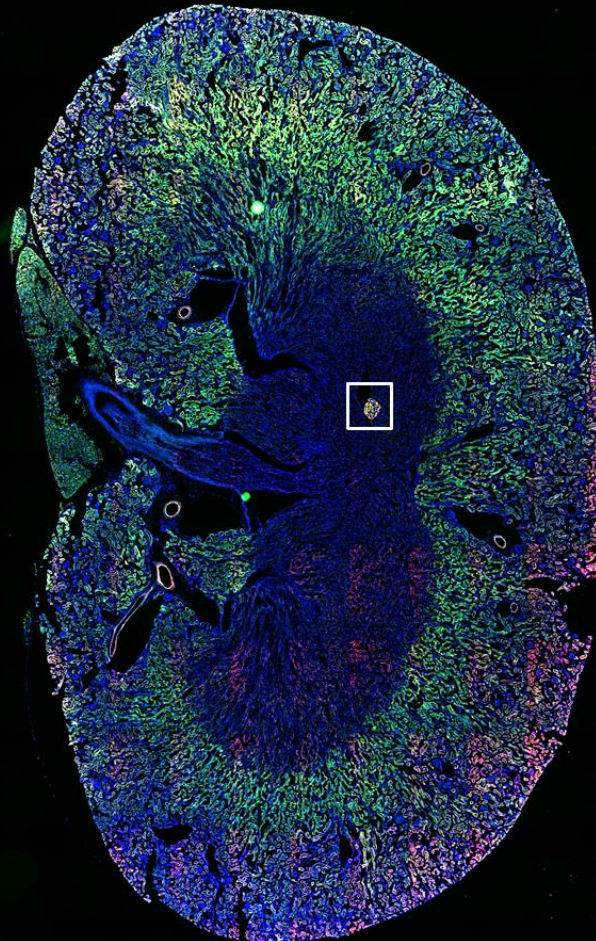
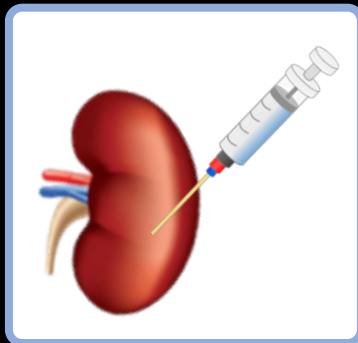


Cell Therapy for Kidney Injury

Intrarenal Transplantation of 3D Cell Spheroids



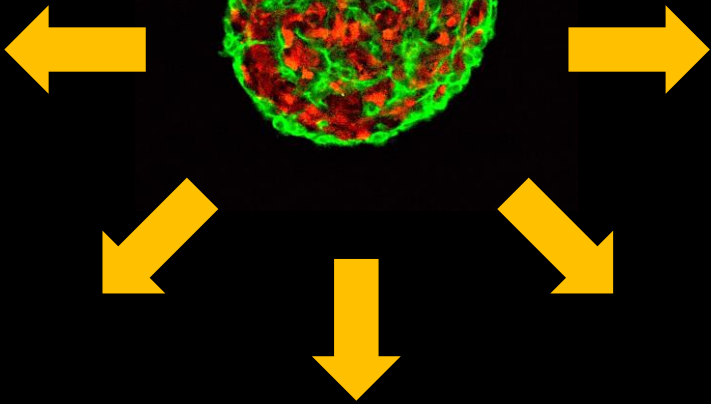
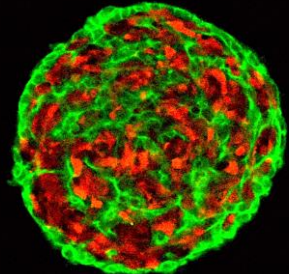
Dr. Hsiang-Hao Hsu
Department of
Nephrology, CGMH



Cell Therapy Using 3D Stem Cell Spheroids

Ischemic stroke
Traumatic brain injury

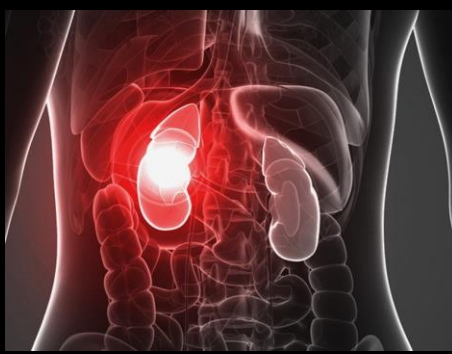
Type I
diabetes mellitus



Peripheral nerve injury

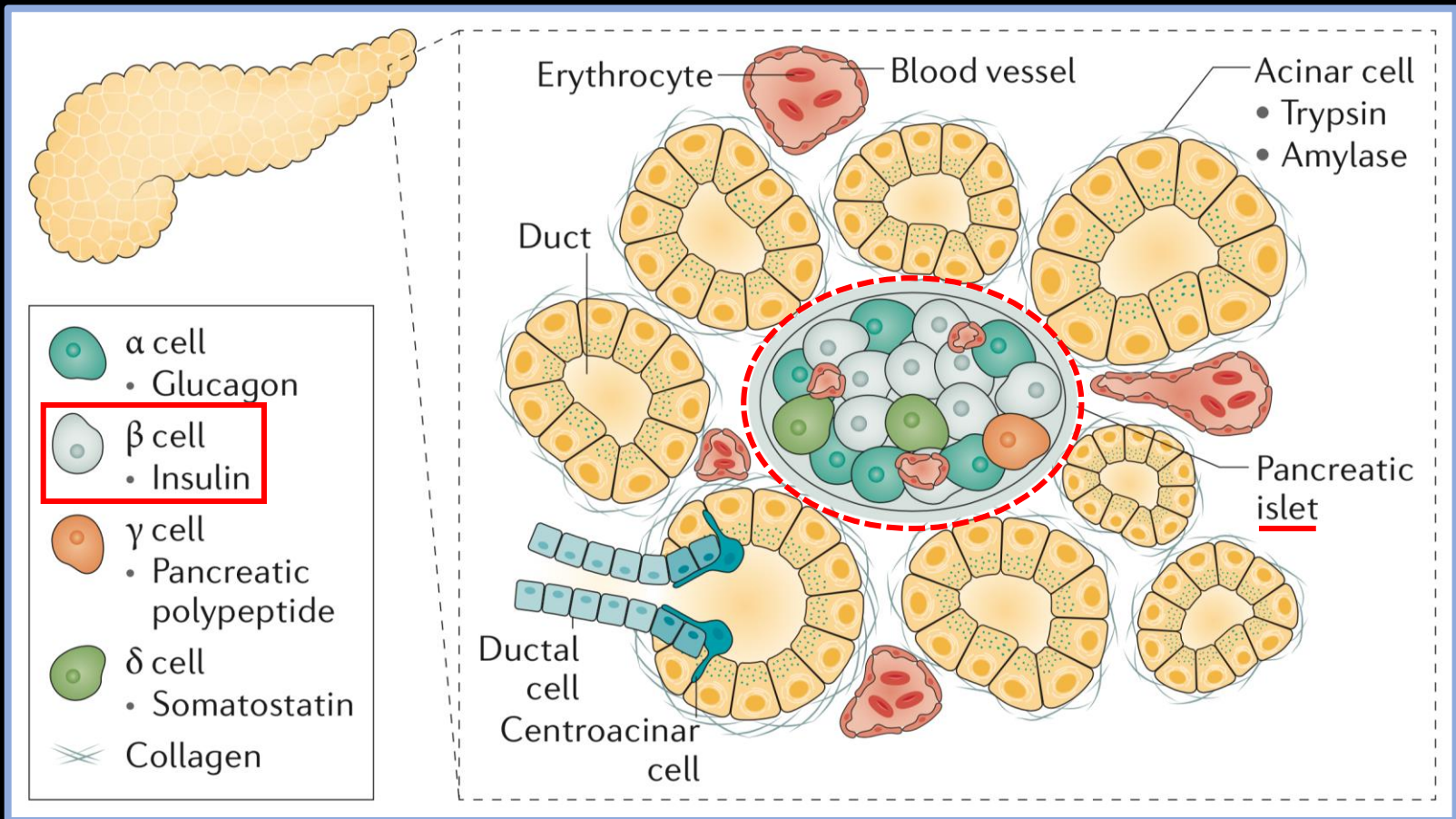
Kidney injury

Tendon/ligament
injury



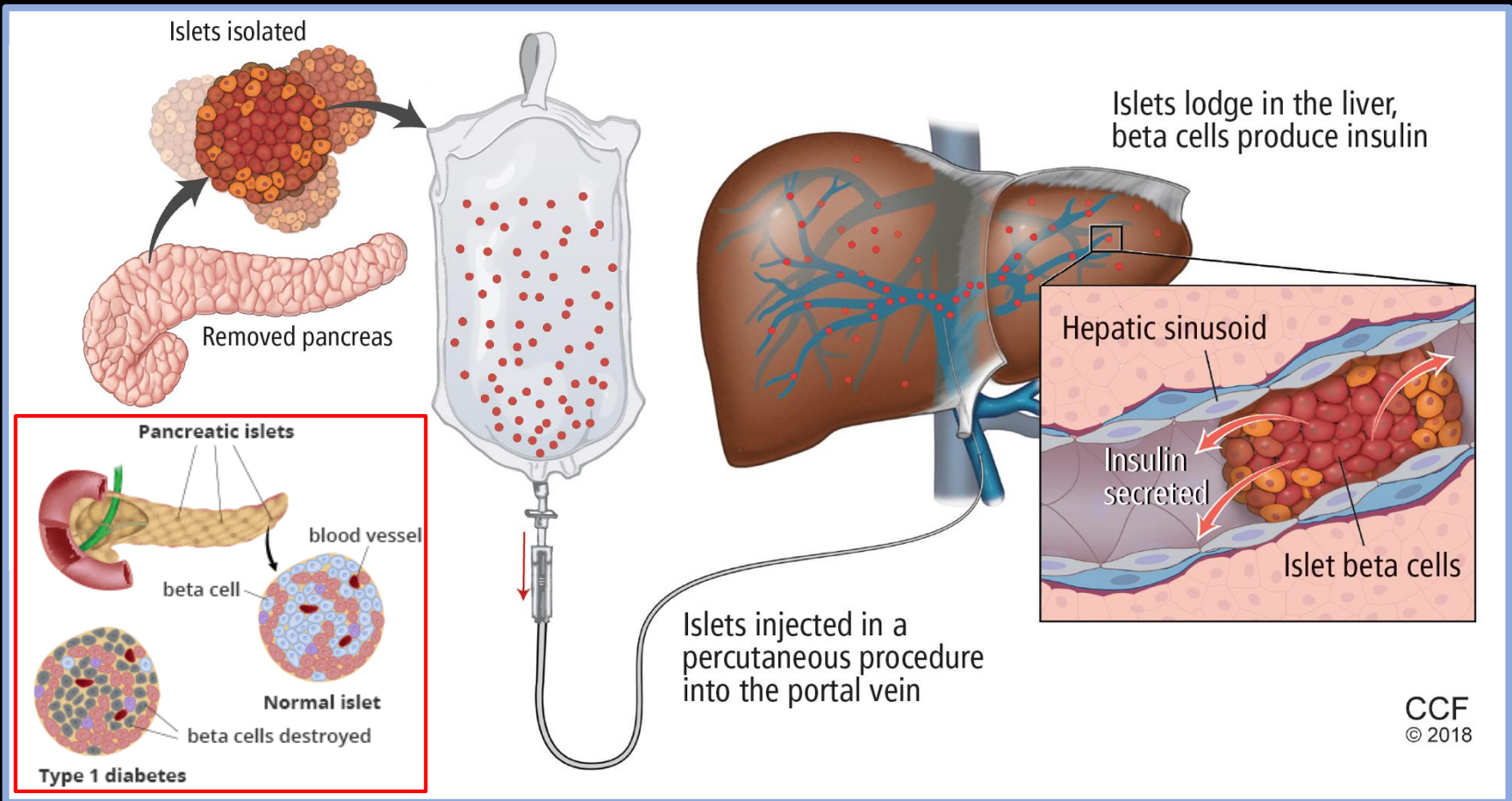
Cell Therapy for Type I DM

Islet, β Cell, and Type I DM



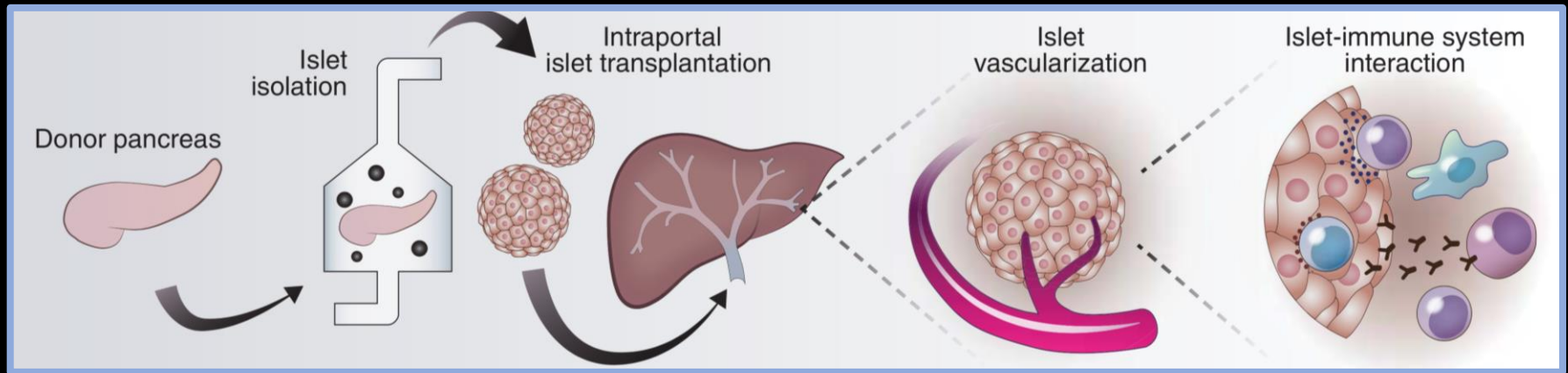
Cell Therapy for Type I DM

Islet Transplantation



Cell Therapy for Type I DM

Islet Transplantation

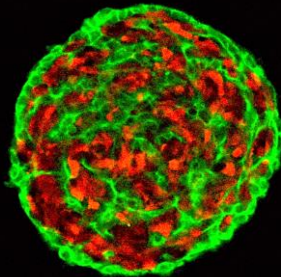
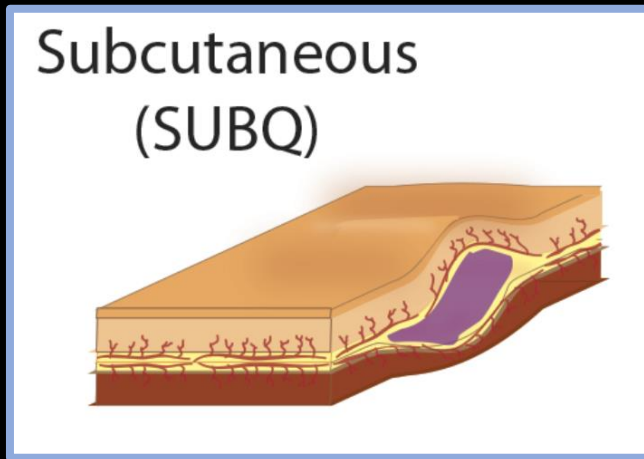


Intraportal transplantation:

- **Invasive**
- Instant **blood**-mediated inflammatory reaction (IBMIR)
- Difficult to **monitor graft**

Cell Therapy for Type I DM

Islet Transplantation



Subcutaneous site:

- Ready access
- Low invasiveness
- Easy monitoring
- High volume capacity
- **Limited vascularization**

3D MSC/HUVEC spheroid:

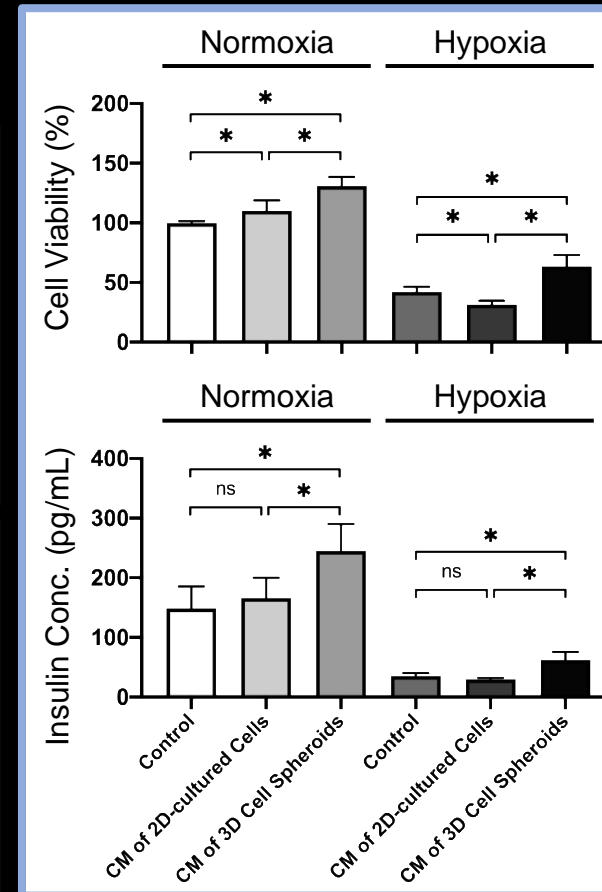
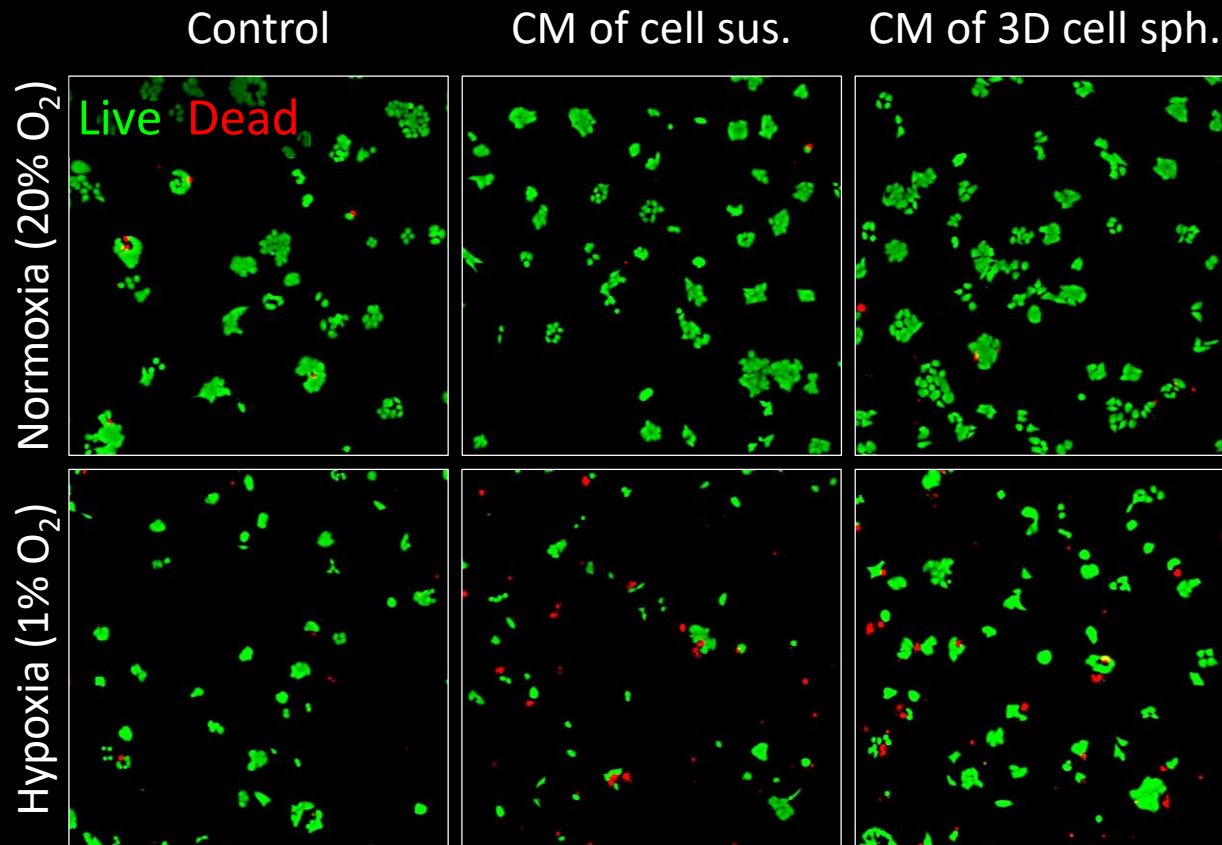
- Pro-angiogenesis
- Anti-apoptosis

Cell Therapy for Type I DM

Pro-survival Potential of 3D Stem Cell Spheroids

Cell: MIN6 cells

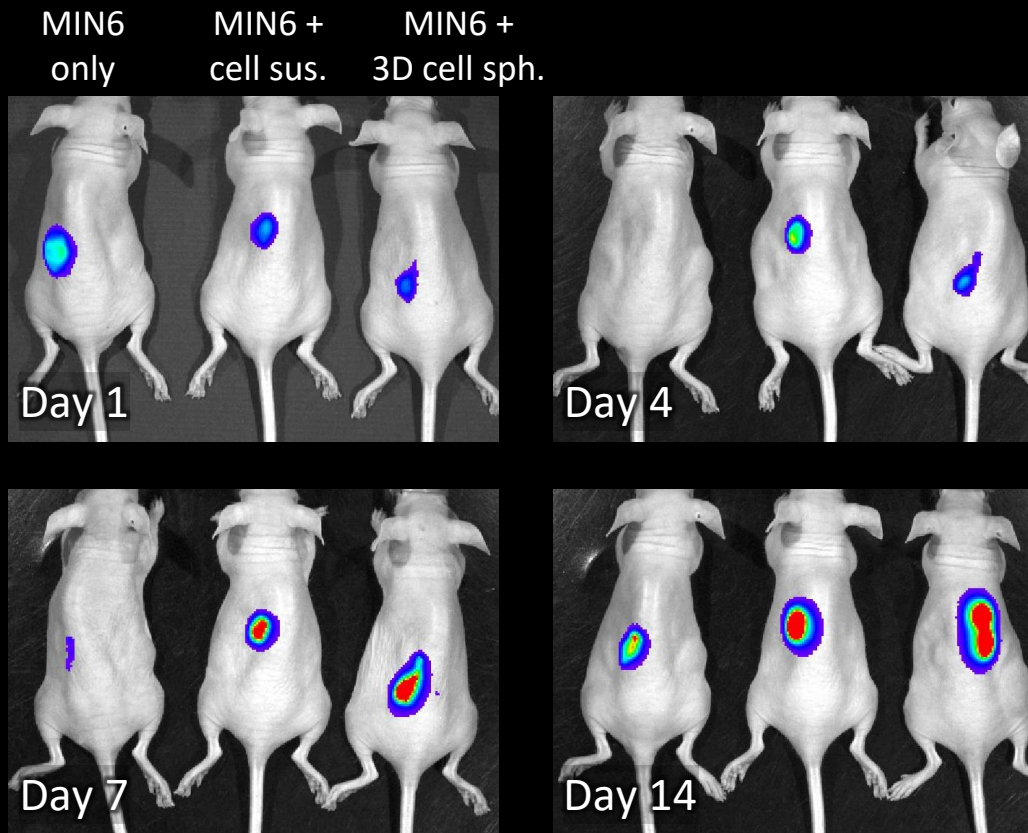
CM: conditioned medium



Cell Therapy for Type I DM

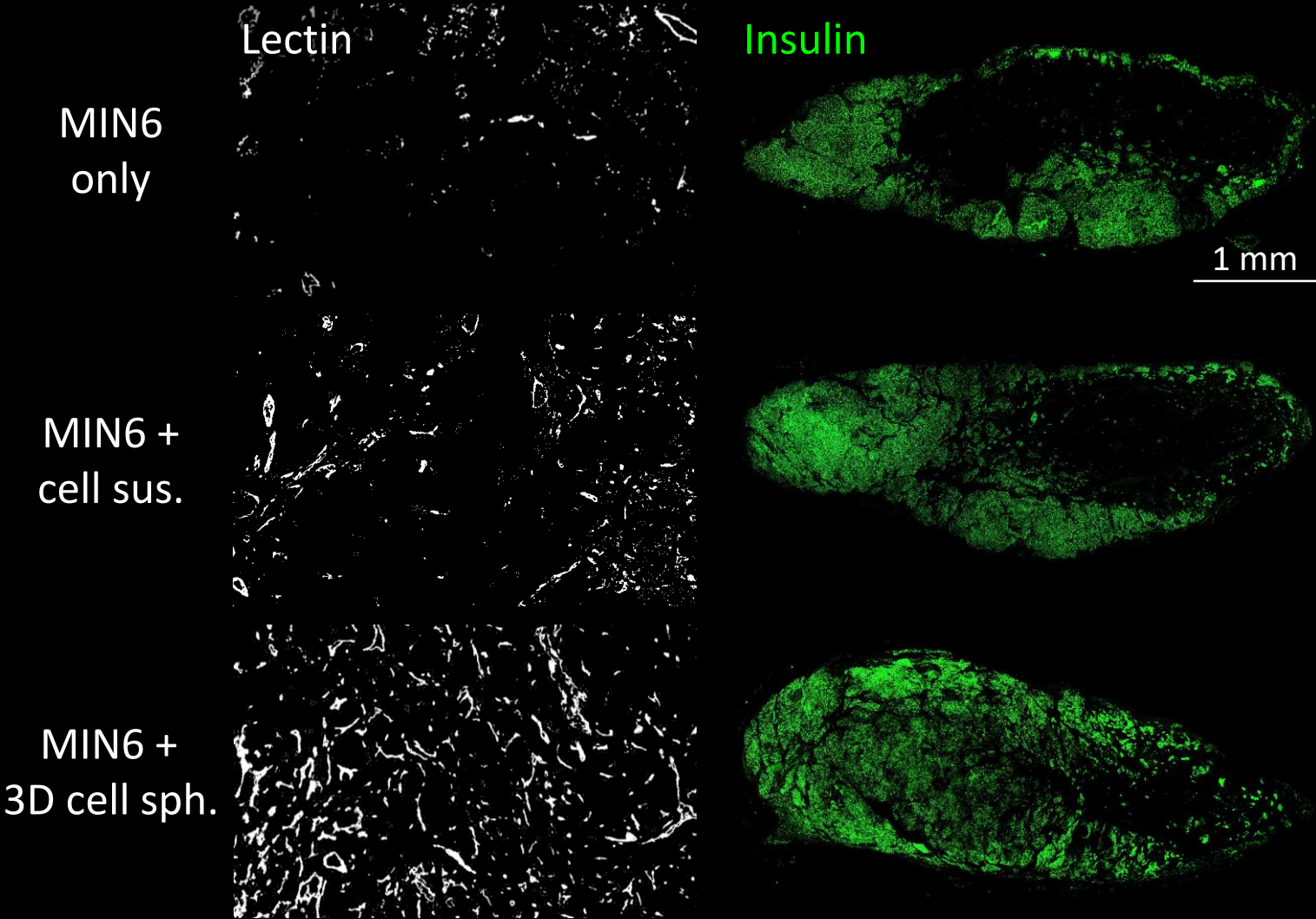
Subcutaneous Transplantation

Cell: MIN6 cells expressing Luciferase



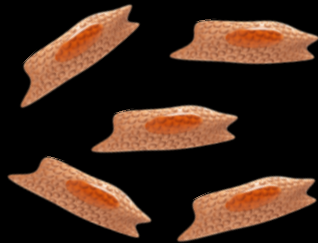
Cell Therapy for Type I DM

Subcutaneous Transplantation



Summary (II)

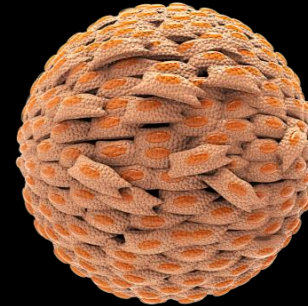
Single cell suspension



Cell only

vs.

3D cell spheroid

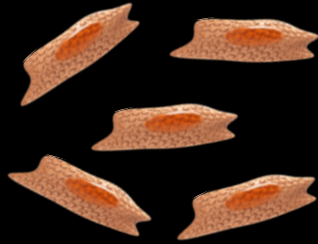


Cell + ECM +
soluble factors

- Transplant therapeutic cells in 3D spheroid configuration shows **superior therapeutic efficacy**

Transplant Therapeutic Cells in 3D Spheroid Configuration

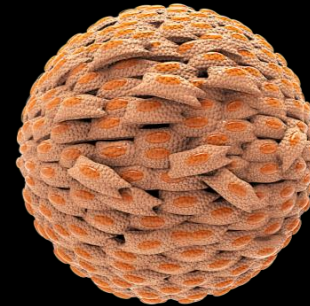
Single cell suspension



Cell only

vs.

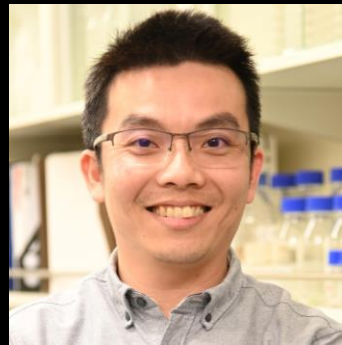
3D cell spheroid



Cell + **ECM** +
soluble factors

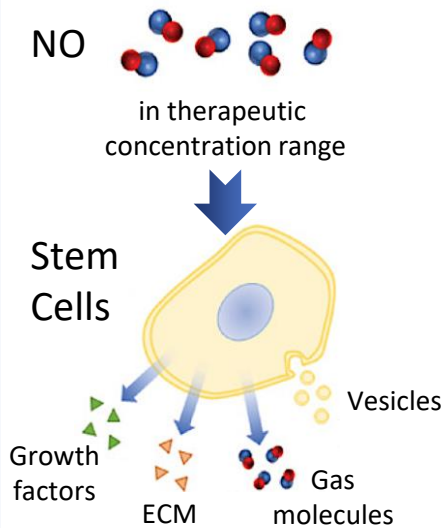
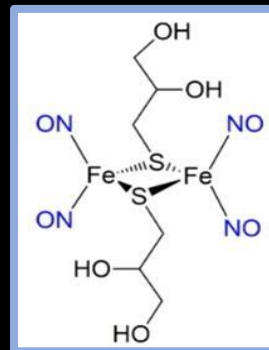
Nitric Oxide (NO)-primed Stem Cells

Enhanced Secretion of Soluble Factors



Prof. Tsai-Te Lu
Institute of BME, NTHU

**Dinitrosyl iron
Complex (DNIC):
long-term NO donor**



Paracrine Secretion

Growth factors
EVs/exosomes

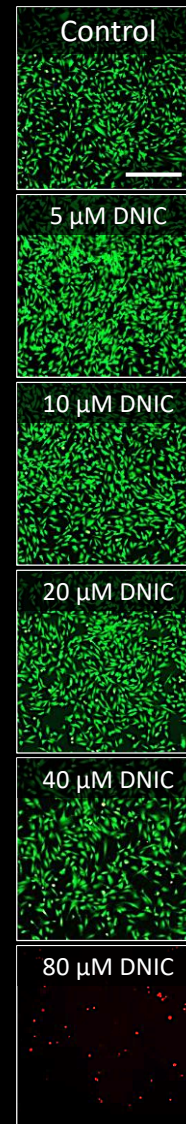
Cell Behavior

Proliferation
Differentiation

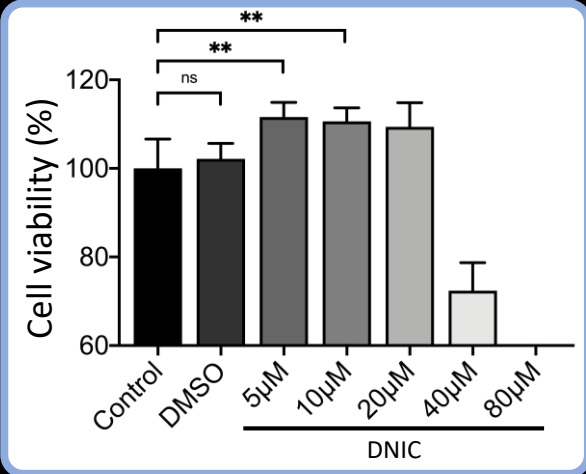
Microenvironment Regulation

Angiogenesis
Neurogenesis
Immunomodulation

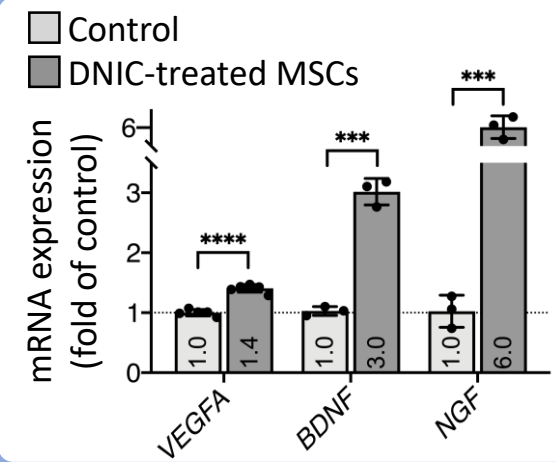
Signaling Pathways



Pro-proliferative effect

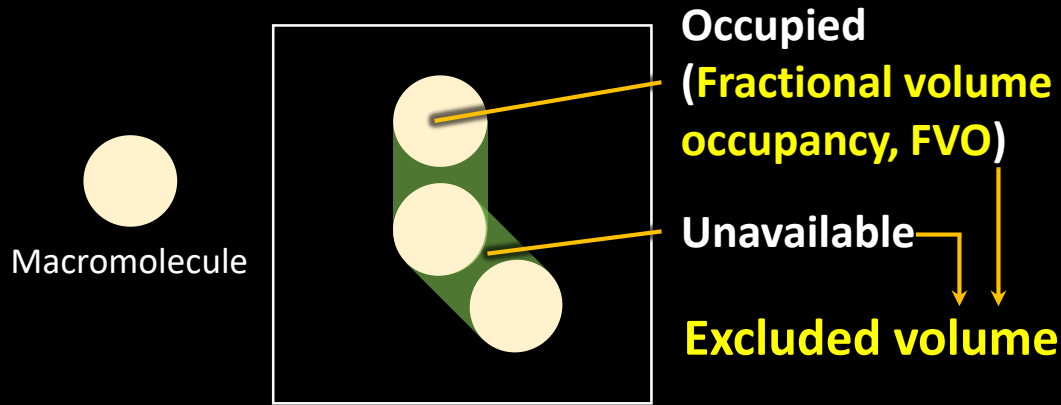


Increased expression of growth factors



Macromolecular Crowding (MMC)

Enhance ECM Deposition



Prof. Anna Blocki
Institute for Tissue Eng. & Reg. Med.,
Chinese University of Hong Kong

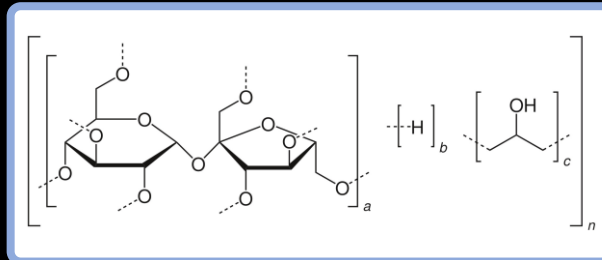
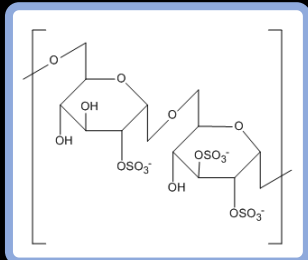
FVO of tissue: **~18%**

FVO of culture medium: **< 6%**

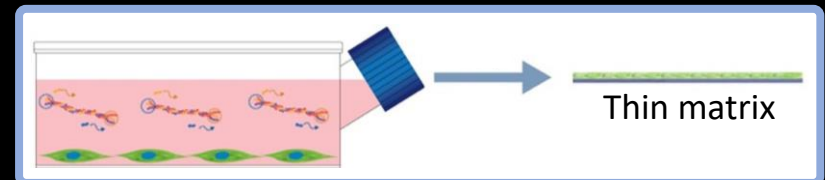
MMC agents: inert macromolecules

Dextran sulfate

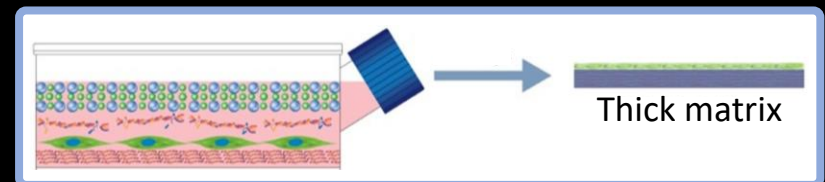
Ficoll



Standard cell culture



With MMC agent

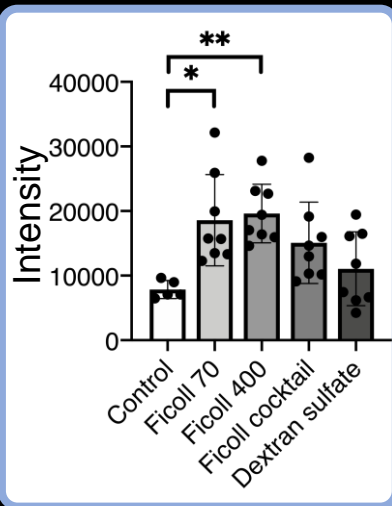
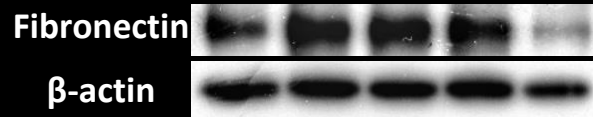


MMC for 3D Cell Spheroid Preparation

Enhance ECM Deposition

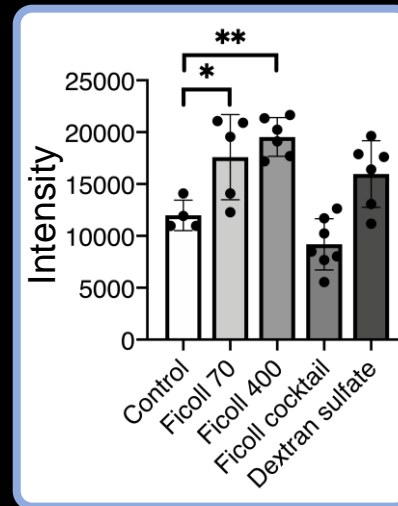
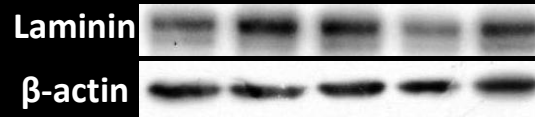
Fibronectin

Control
Ficoll 70
Ficoll 400
Ficoll cocktail
Dextran sulfate

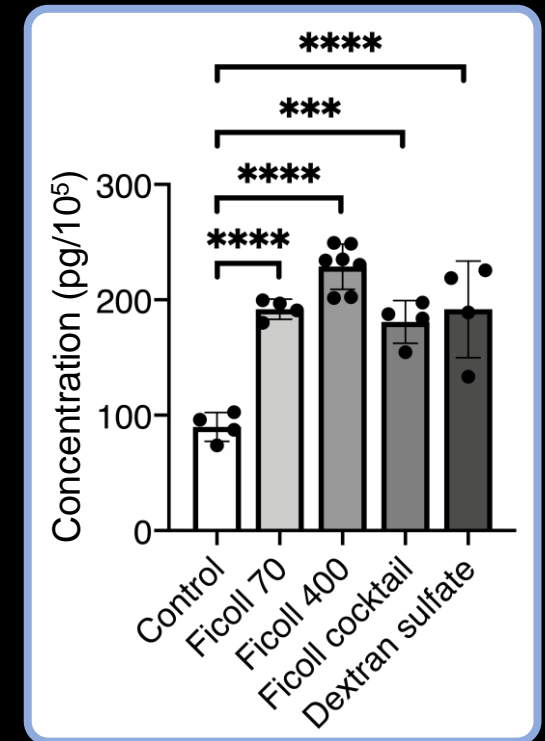


Laminin

Control
Ficoll 70
Ficoll 400
Ficoll cocktail
Dextran sulfate



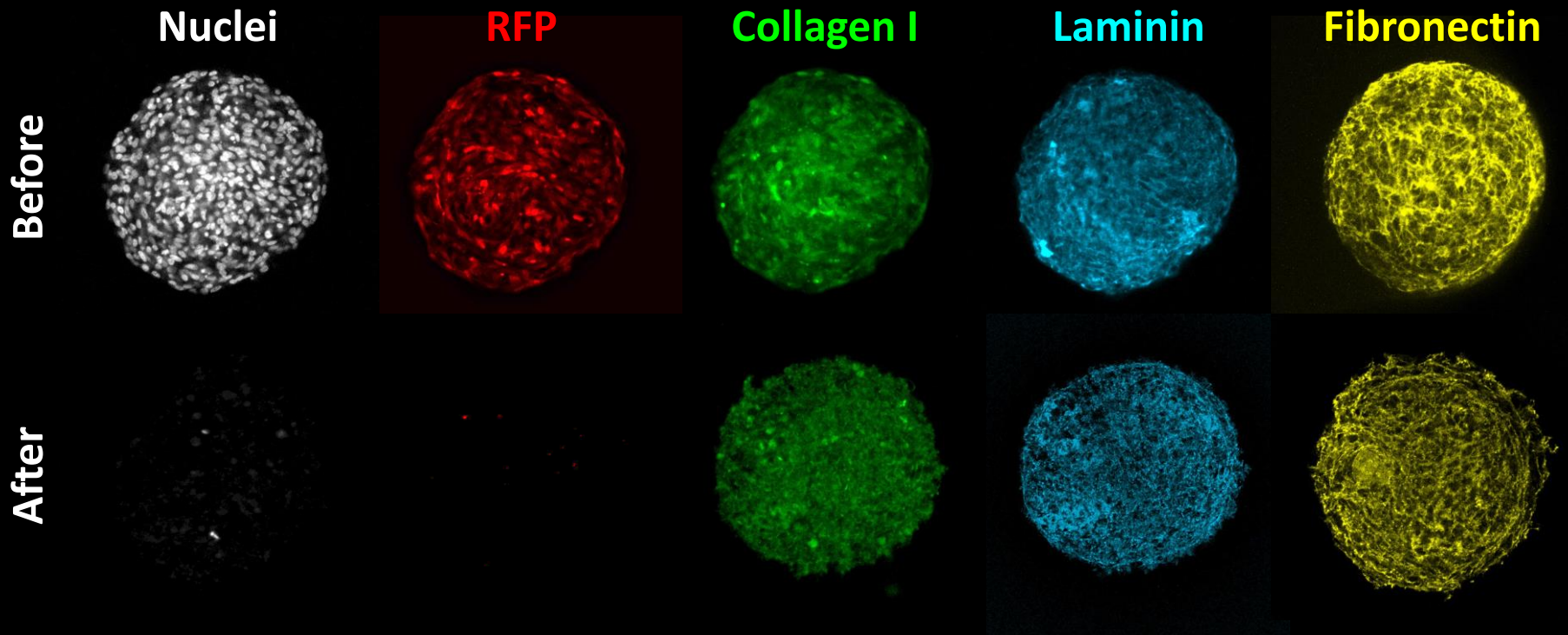
VEGF



Control: 3D cell spheroids prepared without MMC agents

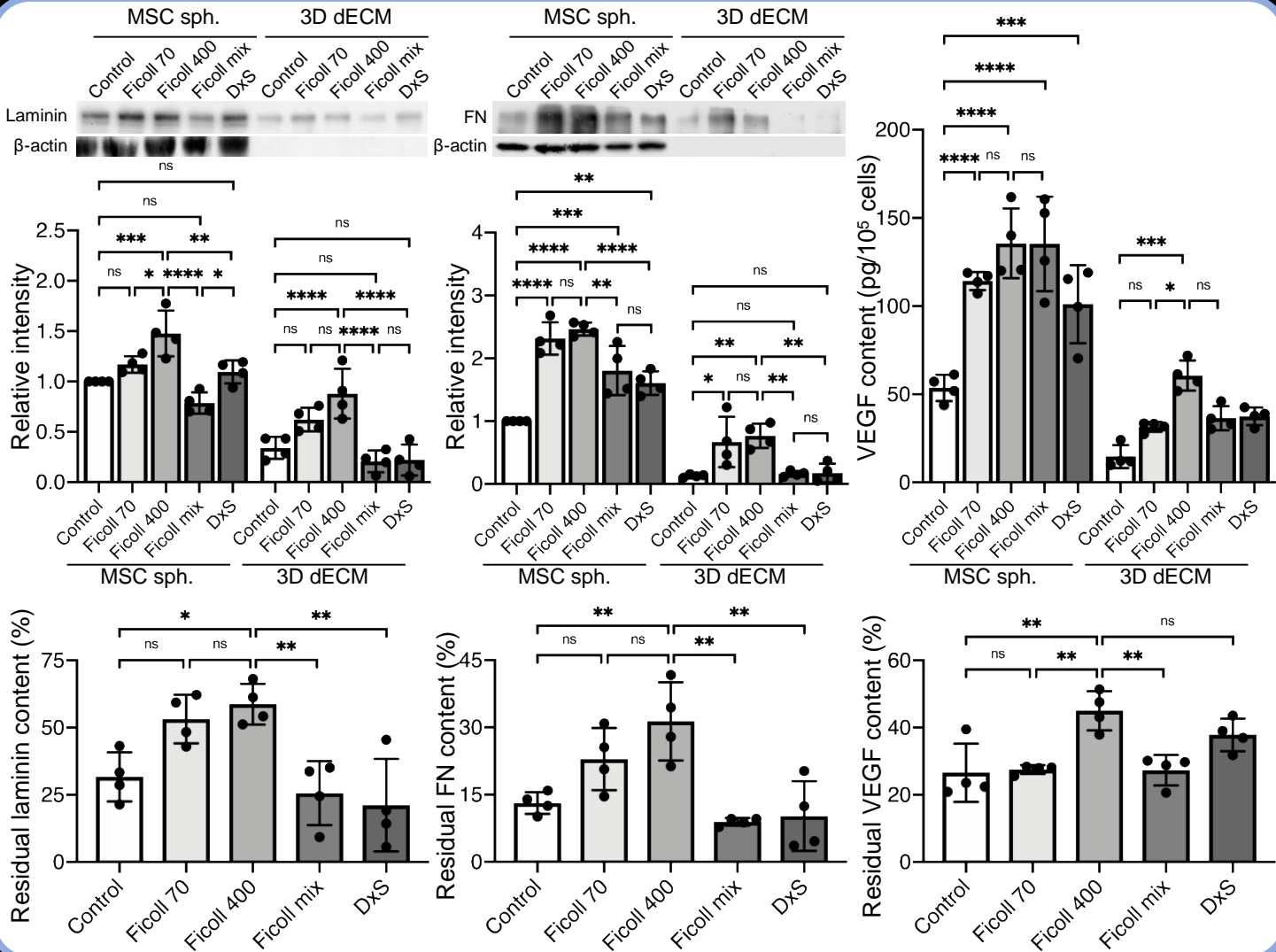
3D Cell Spheroid-derived dECM

Decellularized ECM (dECM)



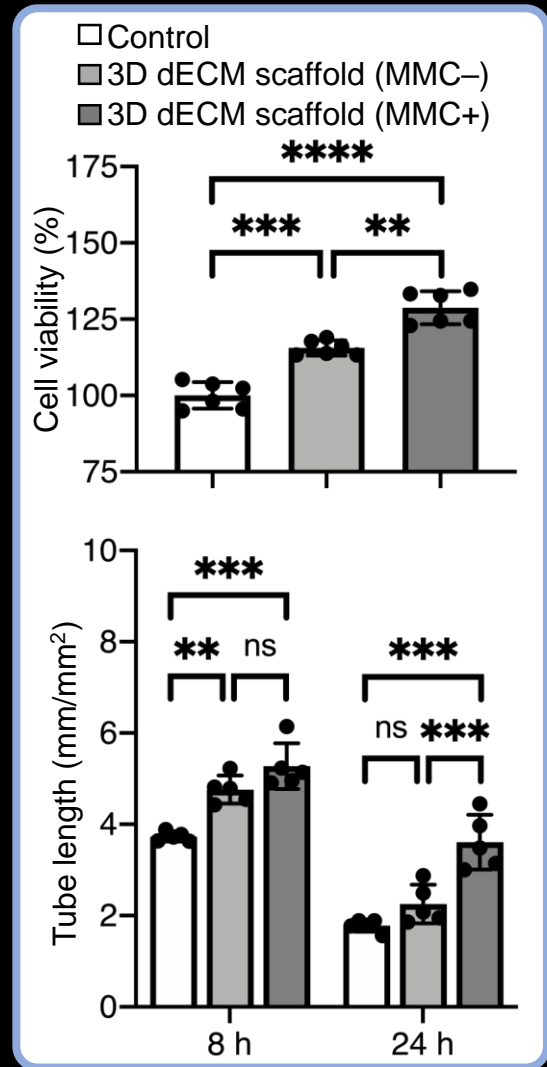
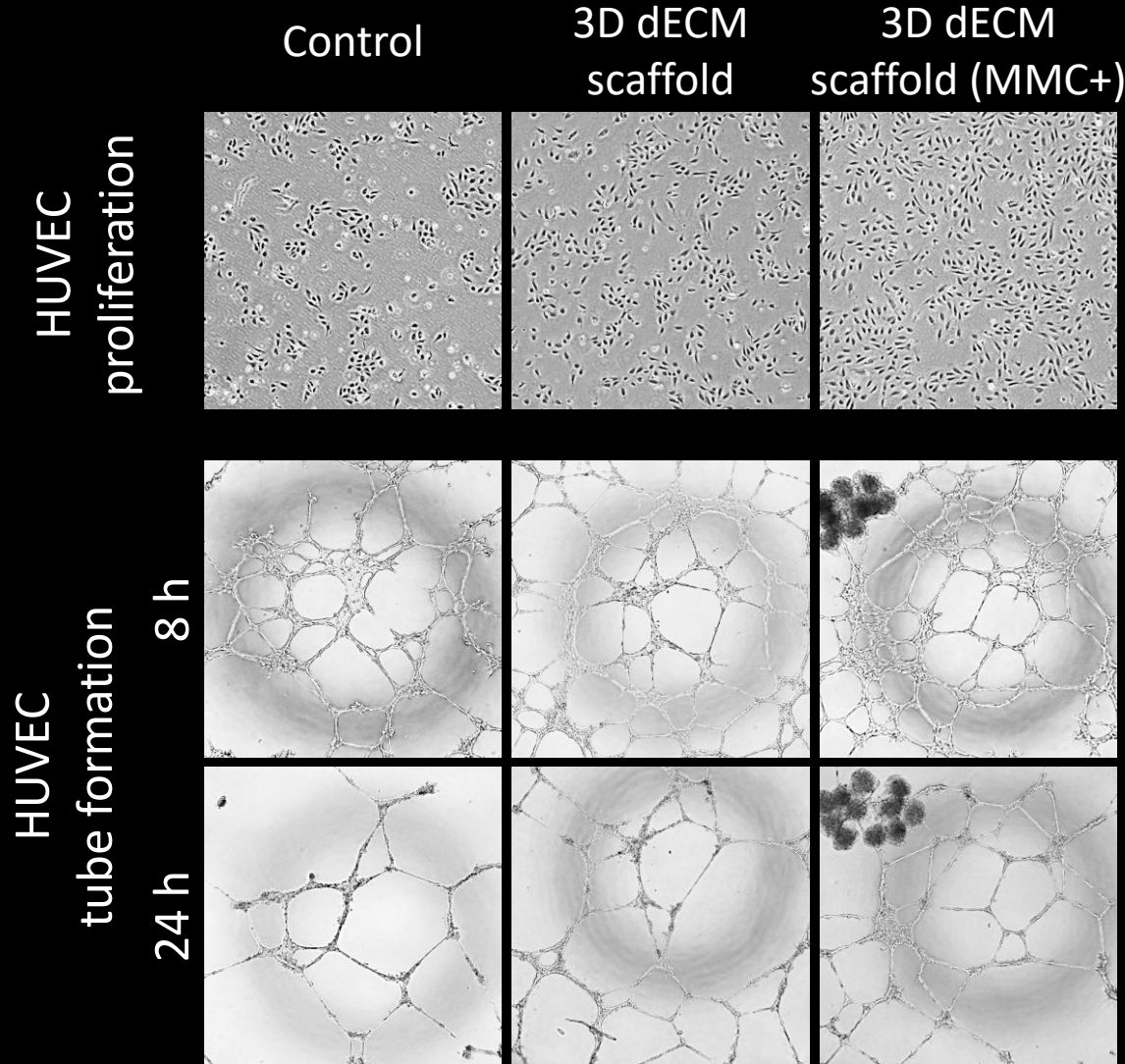
3D Cell Spheroid-derived dECM

Residual ECM & Growth Factors



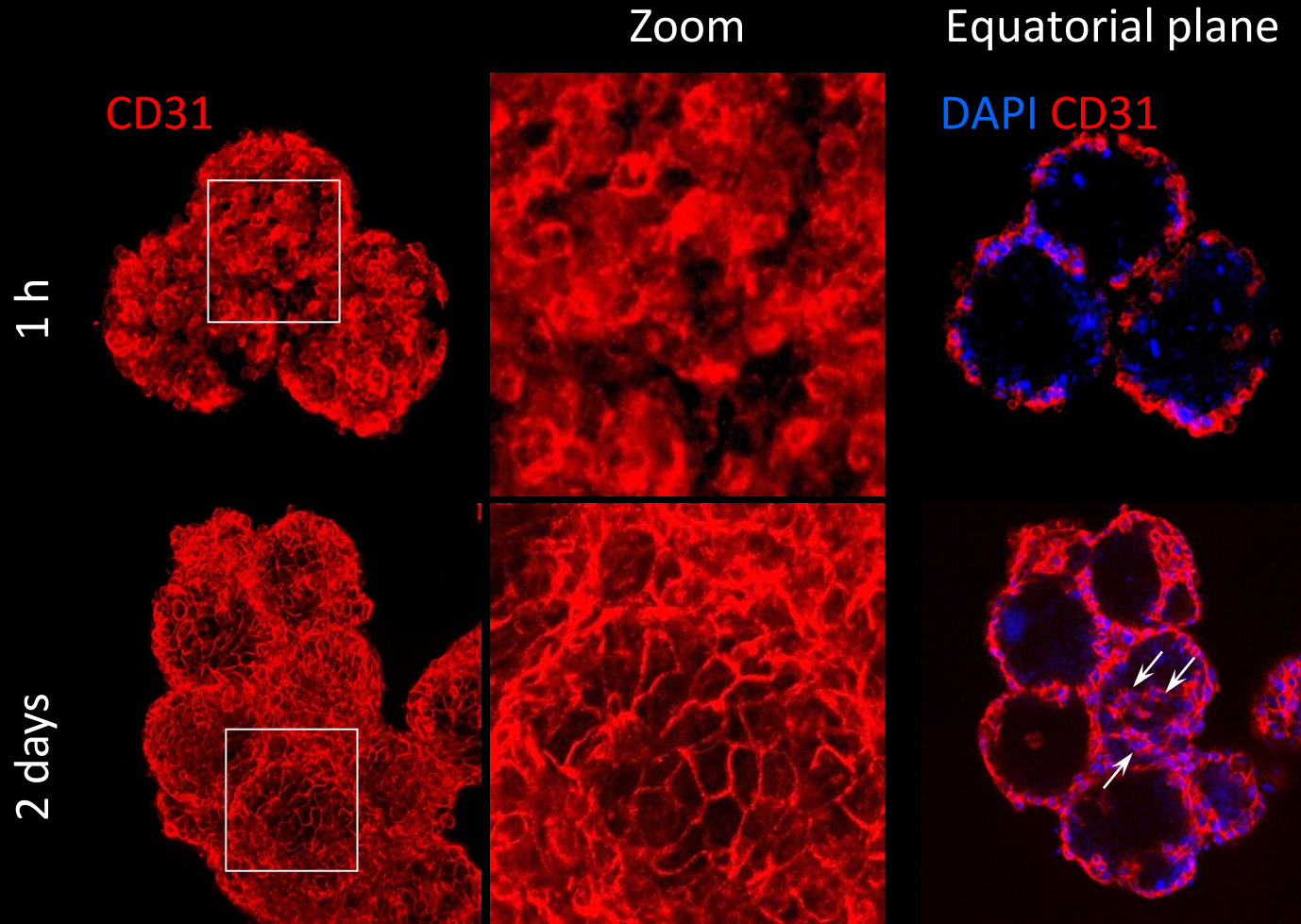
3D Cell Spheroid-derived dECM

Bioactivity of dECM Scaffolds



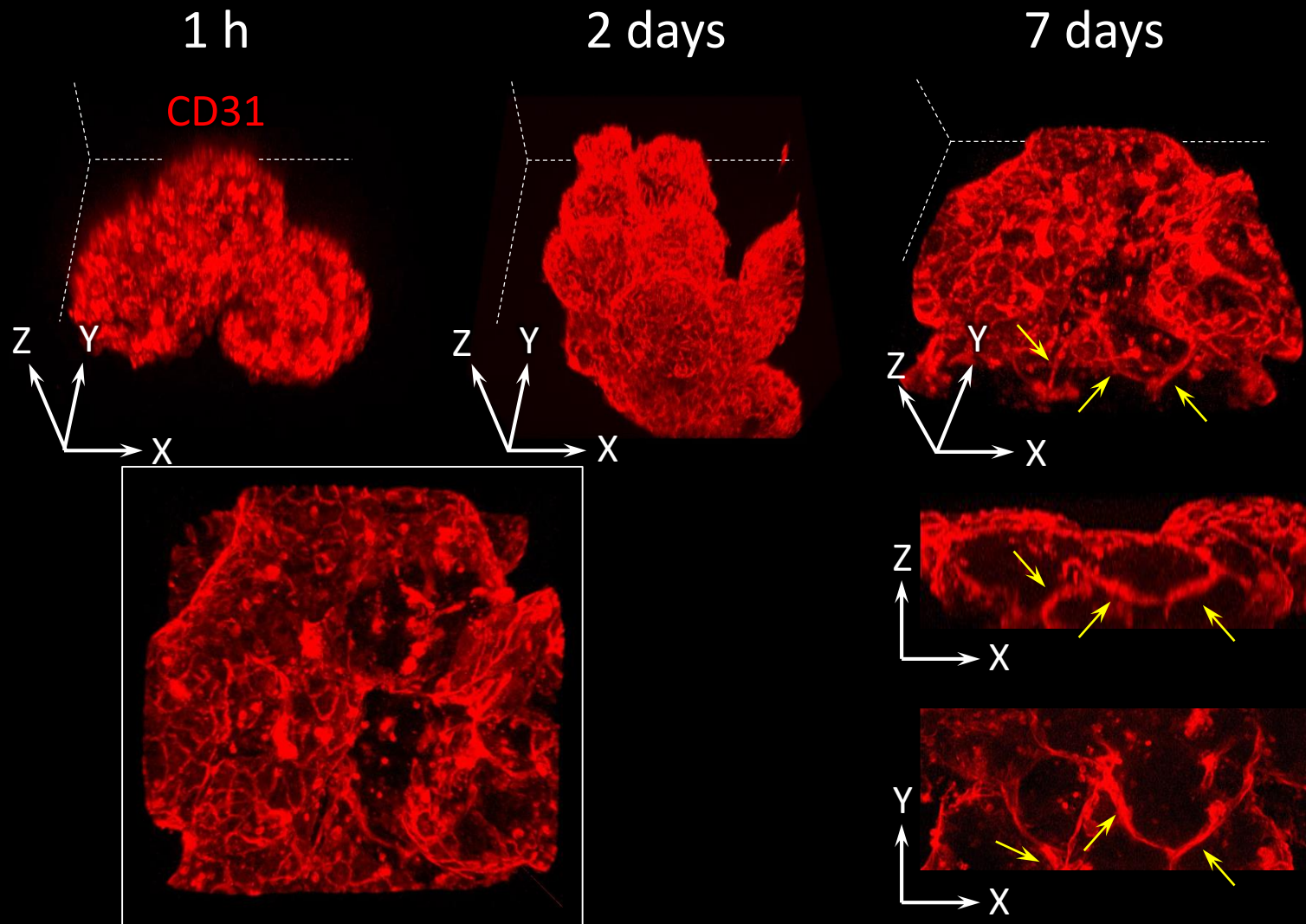
3D Cell Spheroid-derived dECM

dECM as Scaffolds for Tissue Engineering



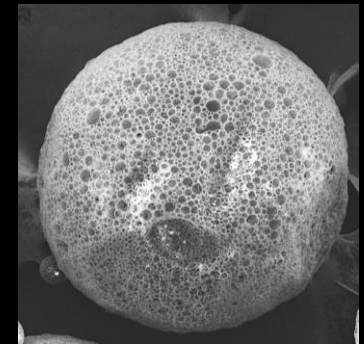
3D Cell Spheroid-derived dECM

dECM as Scaffolds for Tissue Engineering

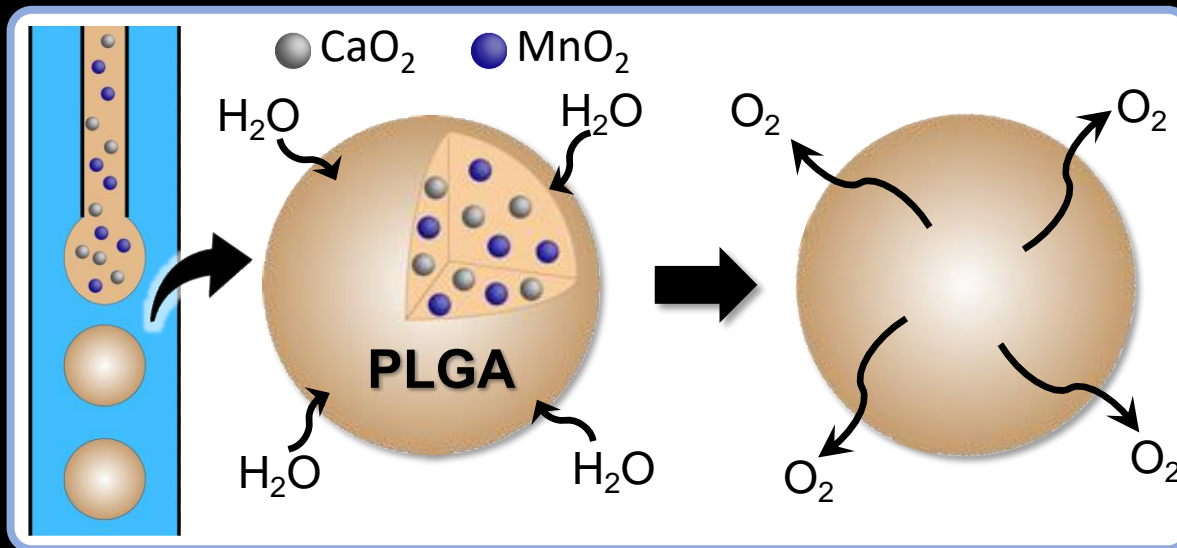
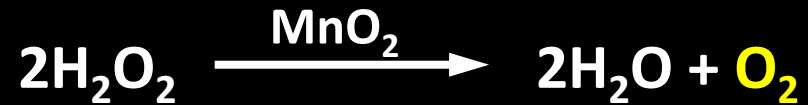


Enhancing Therapeutic Efficacy of Cell Therapy

1. Transplant cells in a 3D spheroid configuration
2. Modulate the **microenvironment** of target tissue with **functional biomaterials**



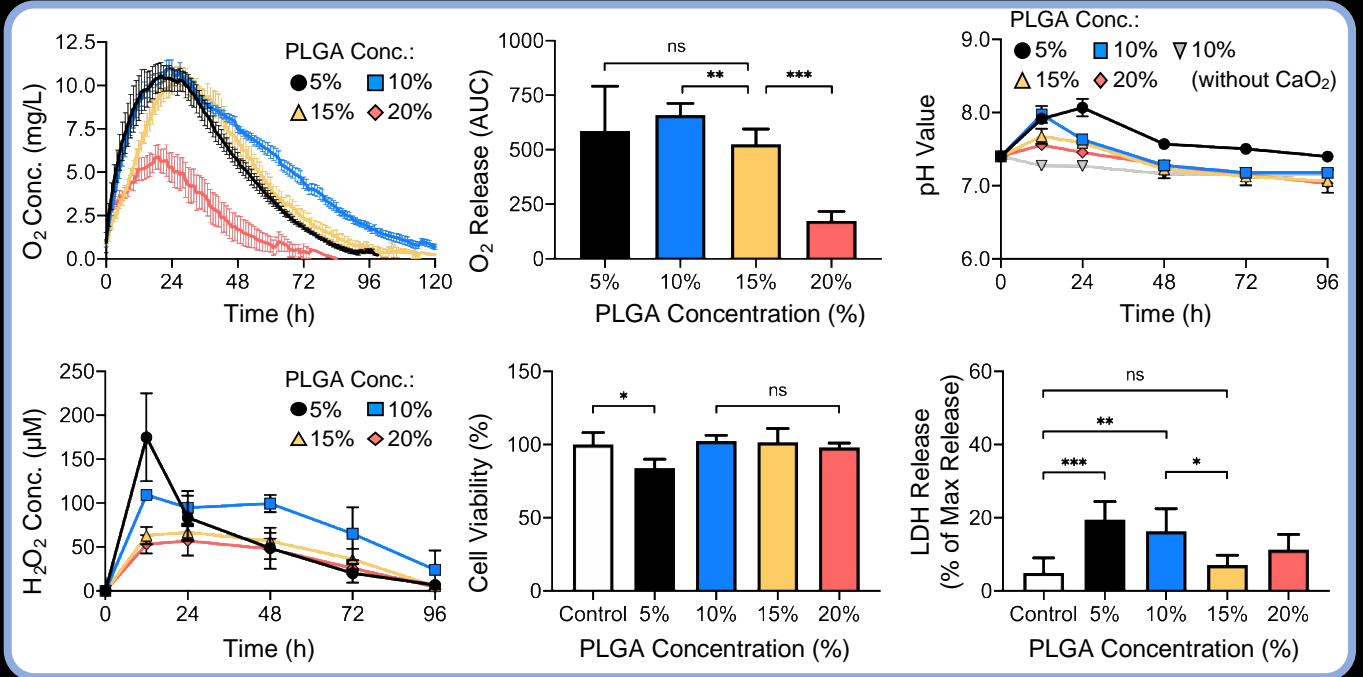
Oxygen-releasing Microparticles (MPs)



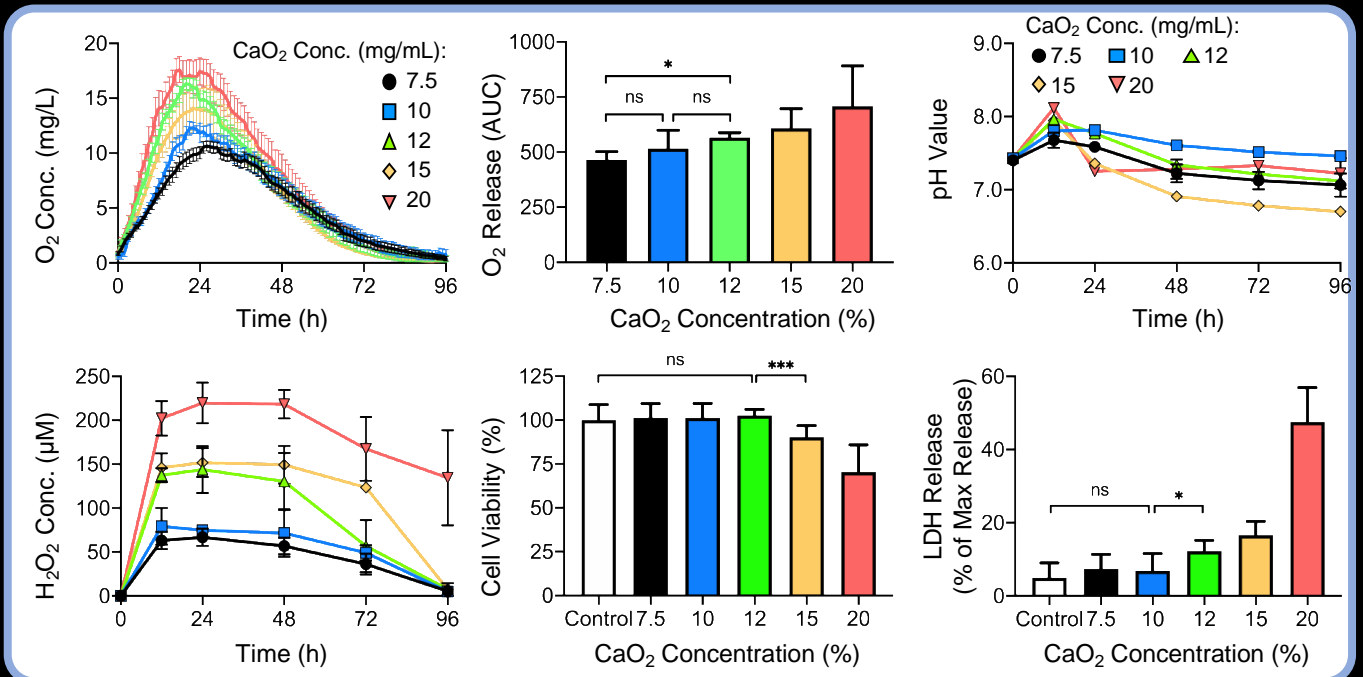
Dr. Po-Liang Lai
Department of Orthopaedic
Surgery, CGMH

Parameter Optimization

PLGA concentration

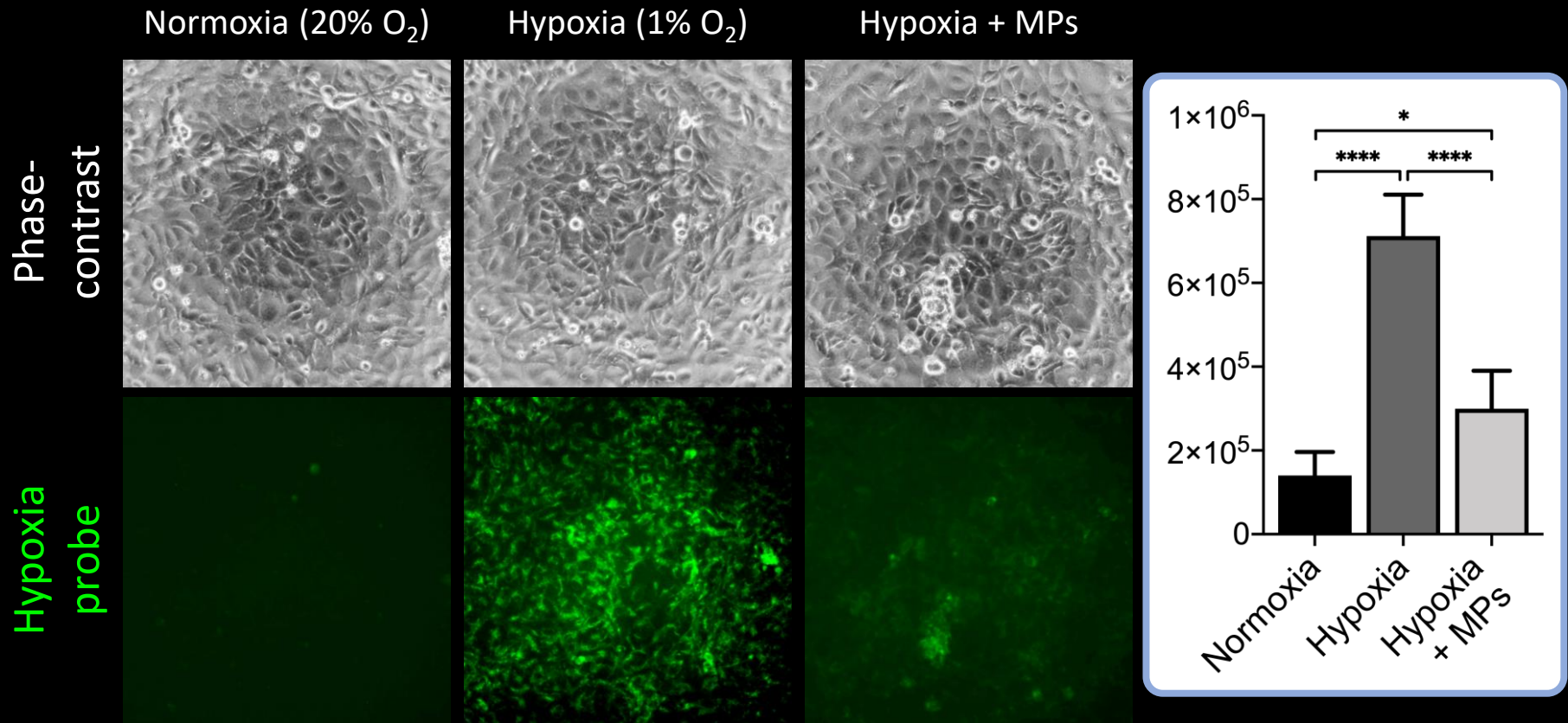


CaO₂ content



Oxygen-releasing Microparticles (MPs)

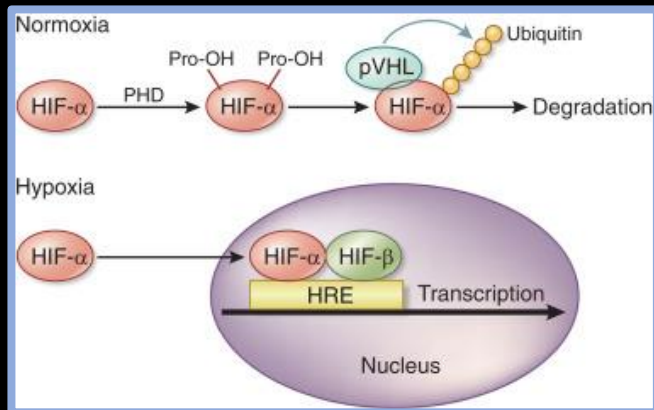
Relieving Cellular Hypoxia



Oxygen-releasing Microparticles (MPs)

Relieving Cellular Hypoxia

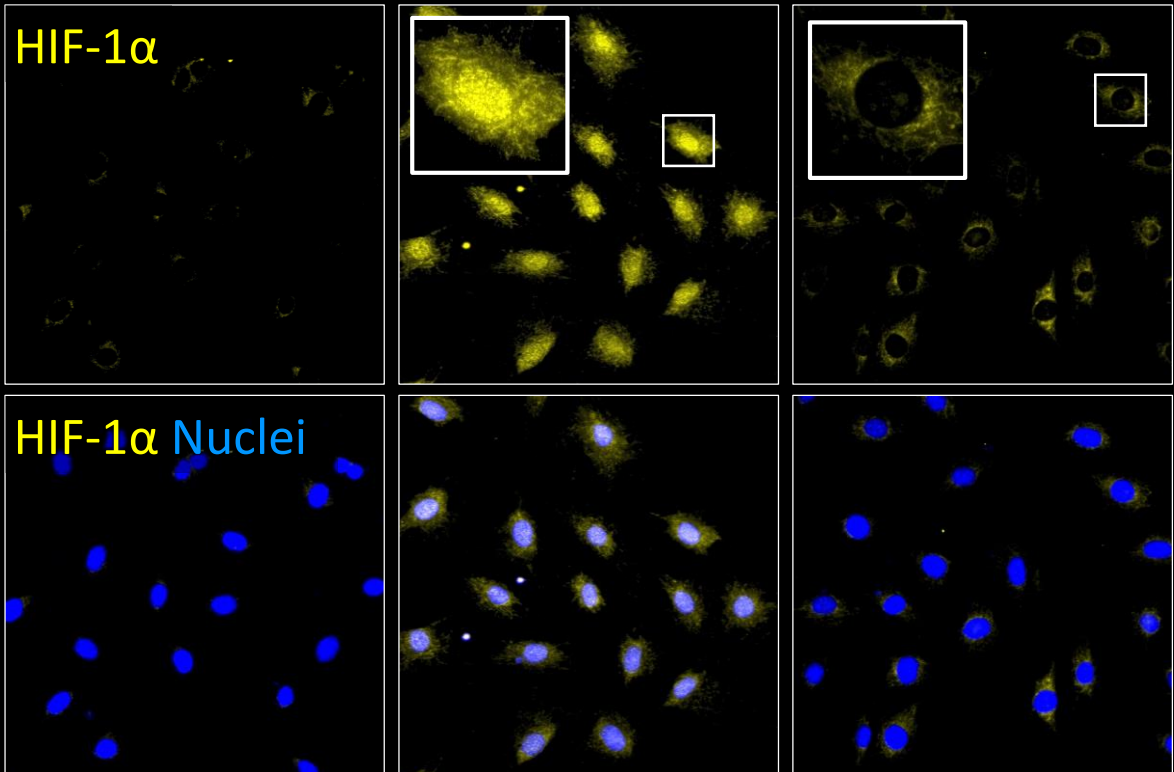
HIF: hypoxia-inducible factor



Normoxia (20% O₂)

Hypoxia (1% O₂)

Hypoxia + MPs



HIF-1 α

HIF-1 α Nuclei

Oxygen-releasing Microparticles (MPs)

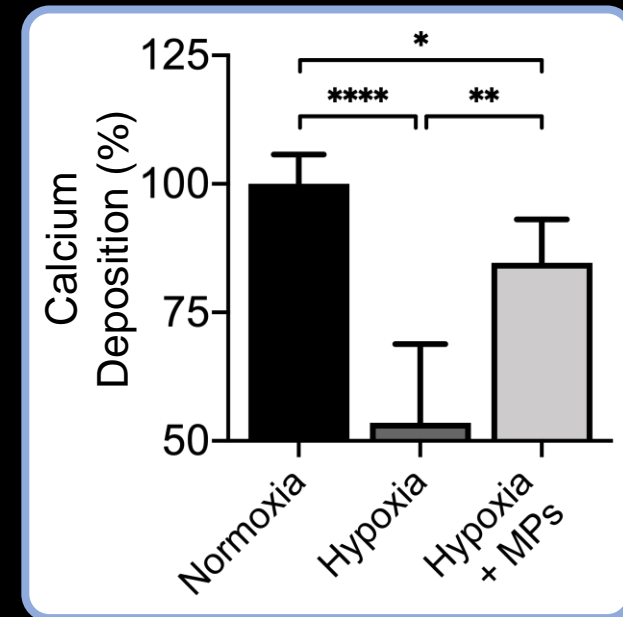
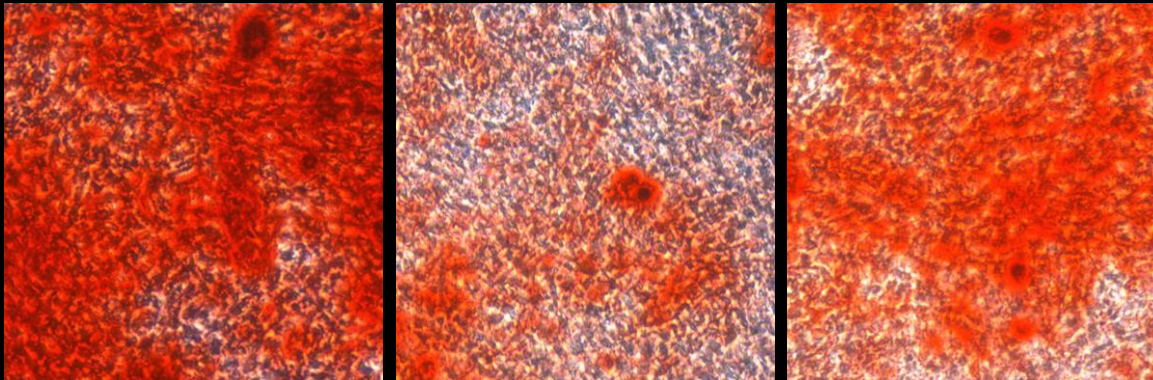
Enhancement of Osteogenesis under Hypoxia

Alizarin Red Staining

Normoxia (20% O₂)

Hypoxia (1% O₂)

Hypoxia + MPs



Scaffolds for Regenerating Corneal Endothelium



Dr. Hung-Chi Chen
Department of
Ophthalmology, CGMH



Gelatin scaffold

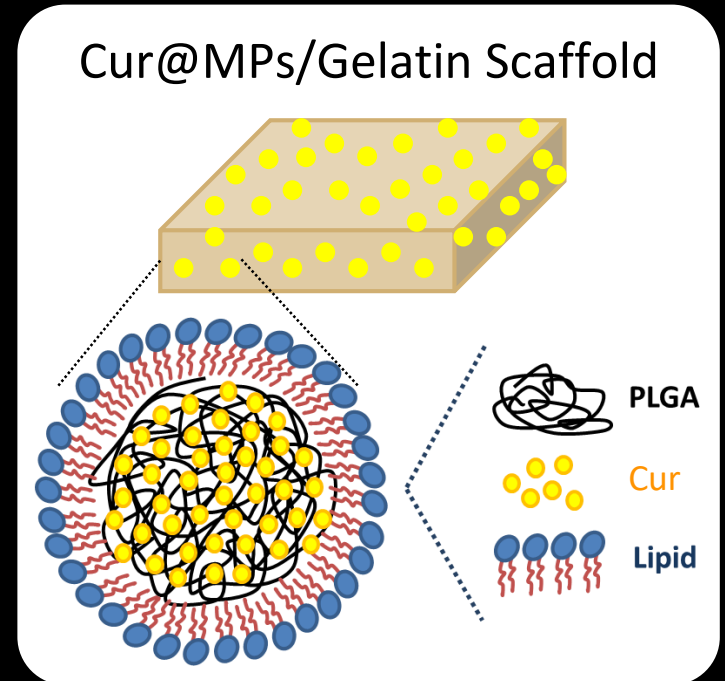
- Transparent
- Support corneal endothelial cells (CECs)

Curcumin

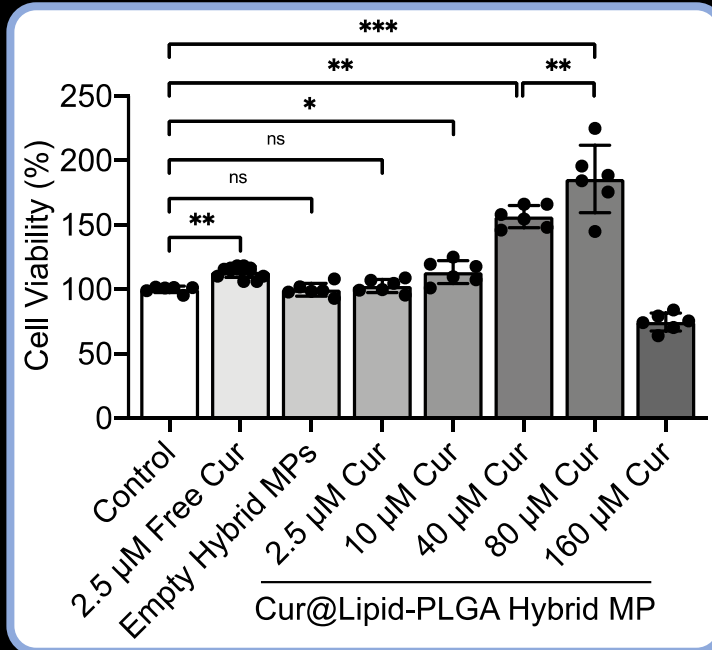
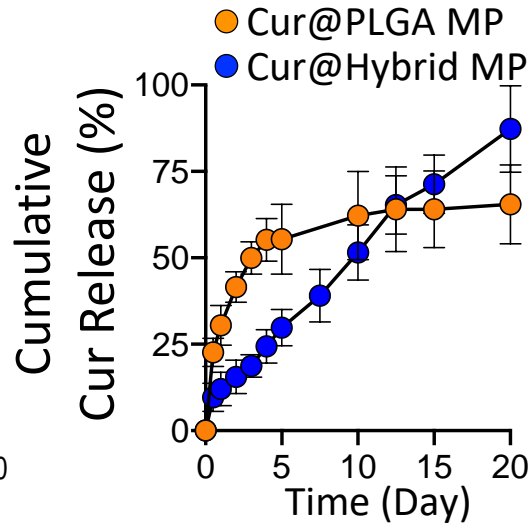
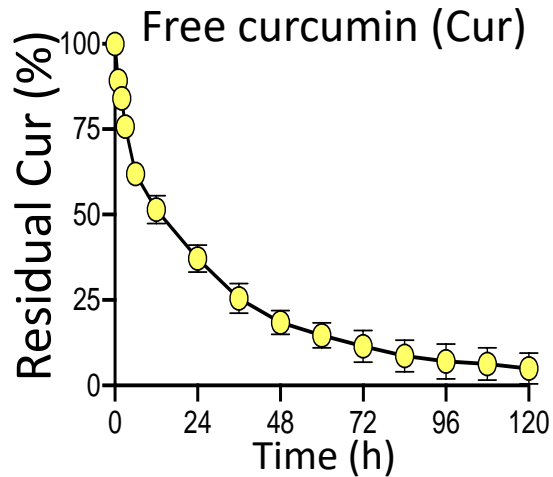
- Anti-oxidant
- Anti-inflammation
- Anti-angiogenesis



Protect engrafted corneal
endothelial cells

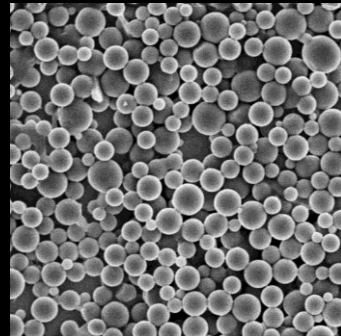
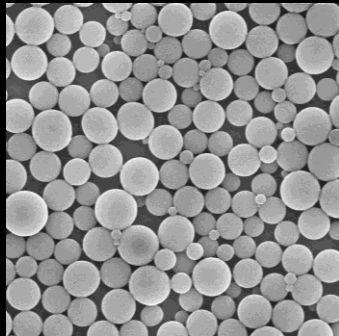


Scaffolds for Regenerating Corneal Endothelium



PLGA MP

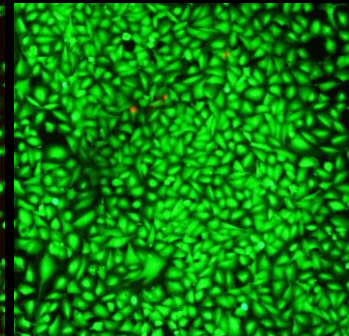
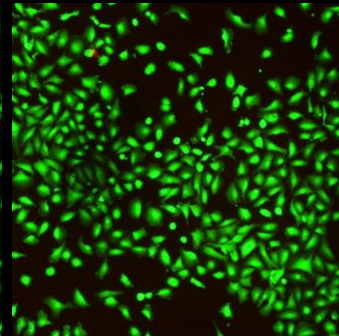
Lipid-PLGA Hybrid MP



Control

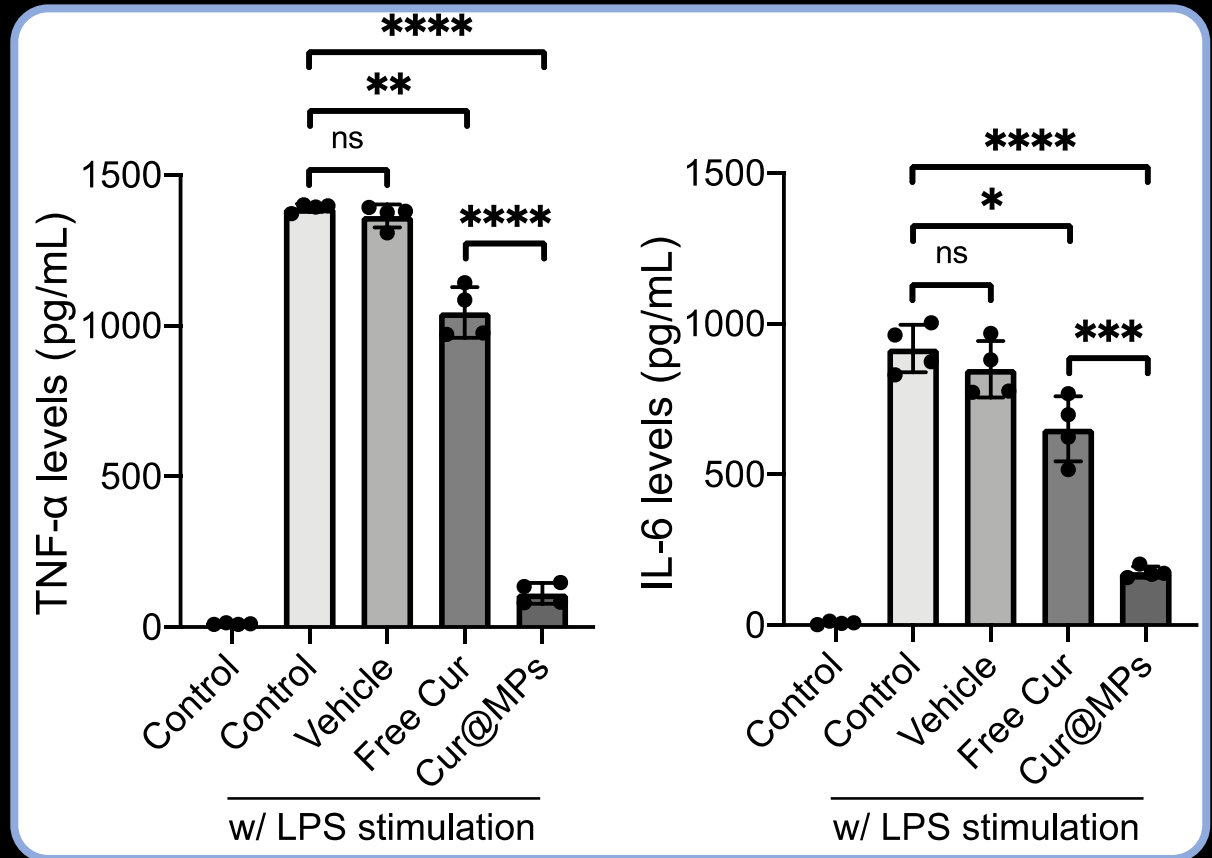
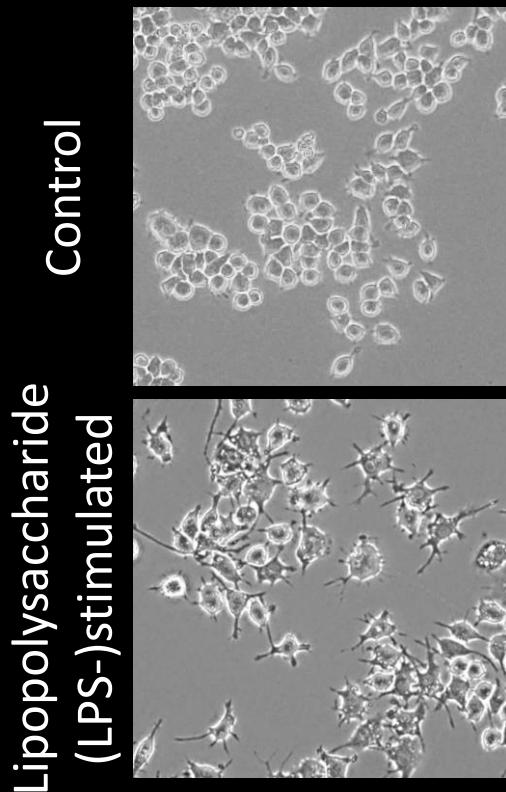
2.5 μM Free Cur

80 μM Cur@MP



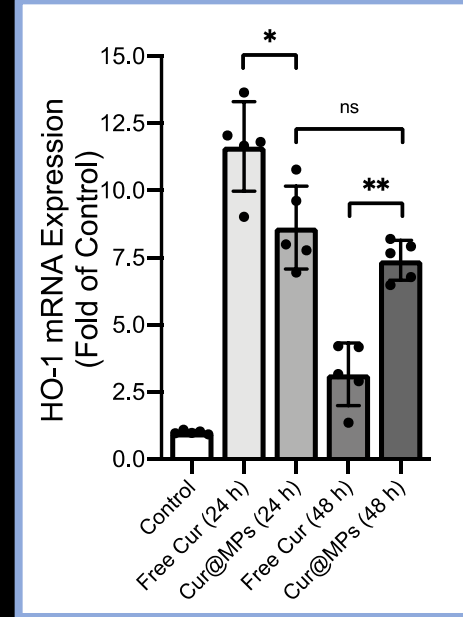
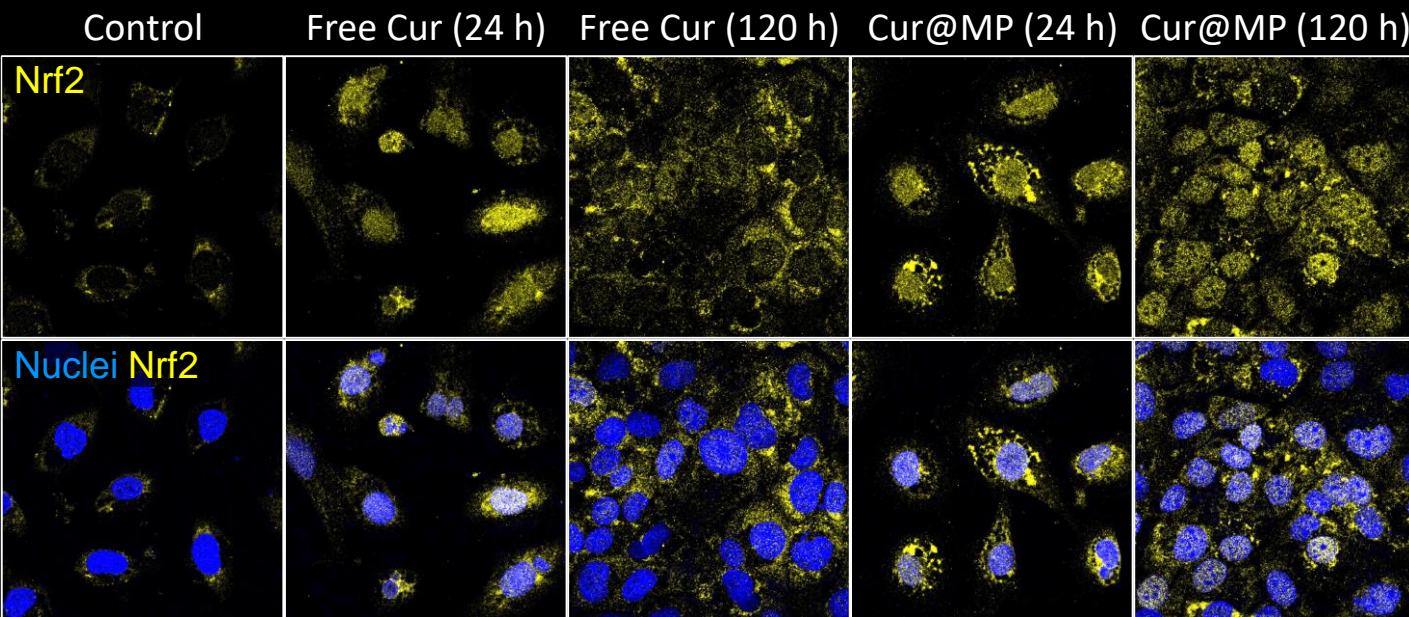
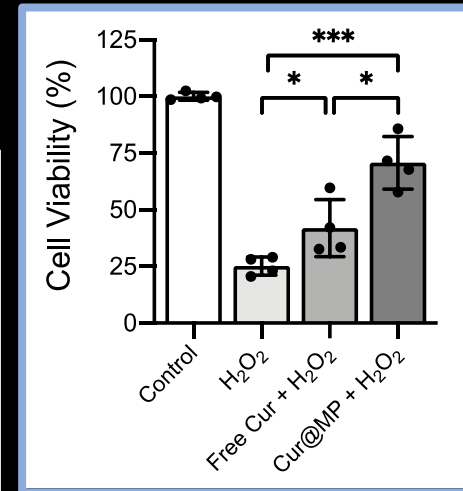
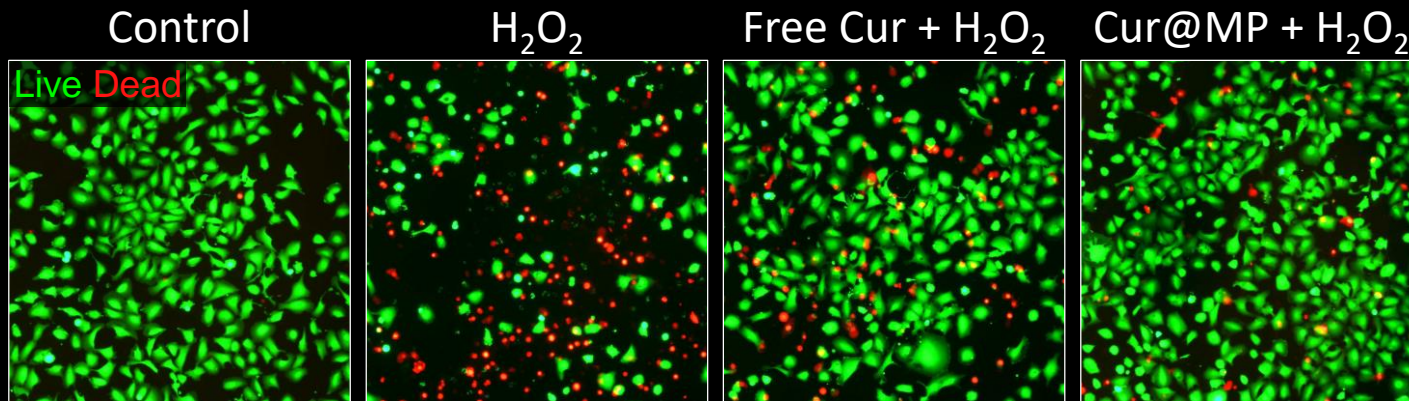
Scaffolds for Regenerating Corneal Endothelium Anti-inflammatory Potential

Raw264.7
macrophage



Scaffolds for Regenerating Corneal Endothelium

Antioxidant Potential



Scaffolds for Regenerating Corneal Endothelium

Anti-angiogenic Potential

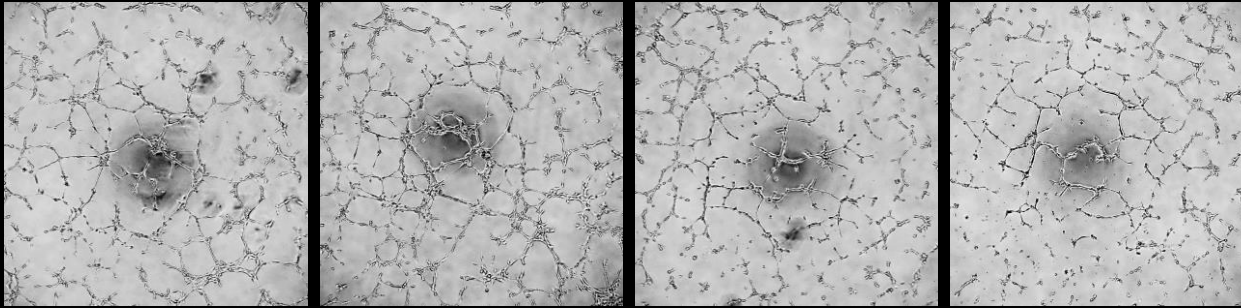
Control

Vehicle

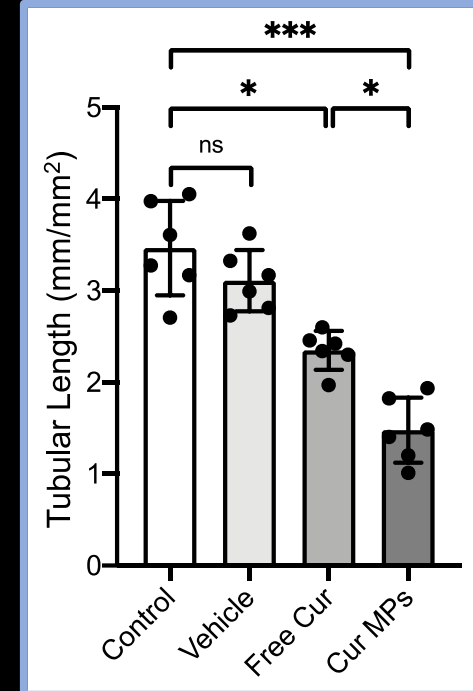
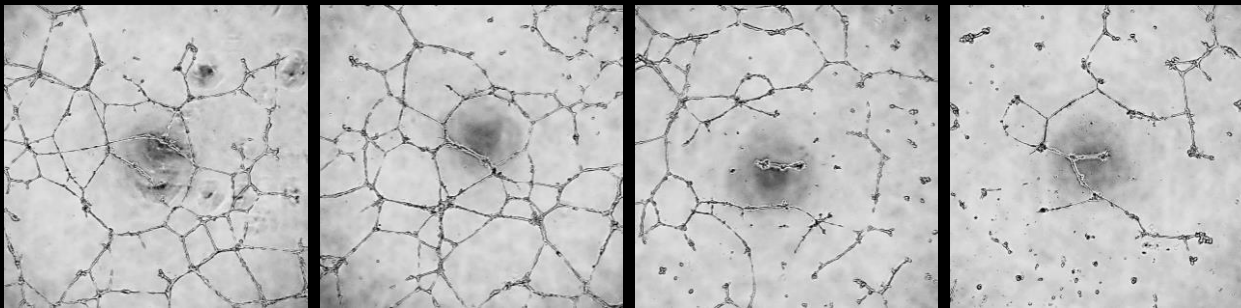
Free Cur

Cur@MP

8 h

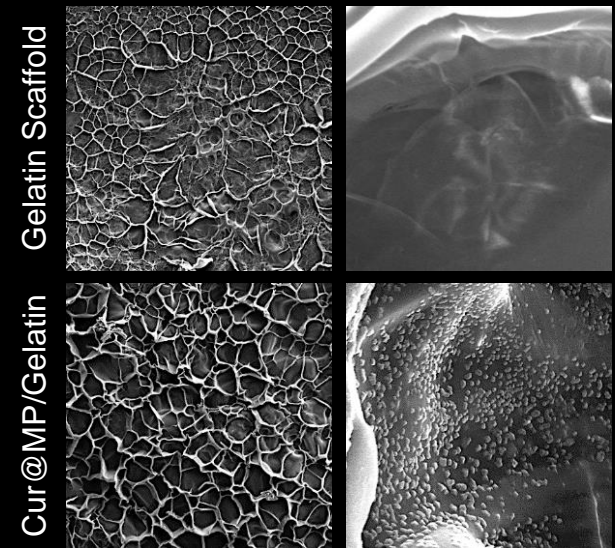
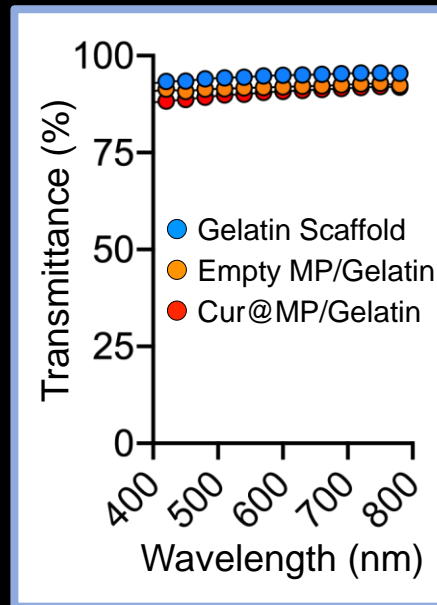
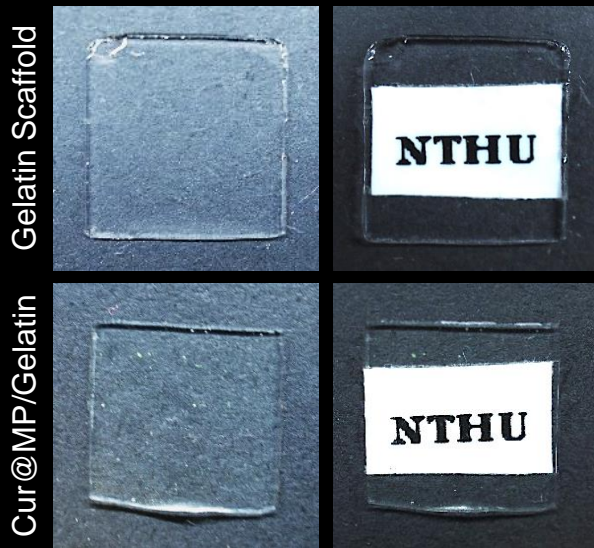


24 h



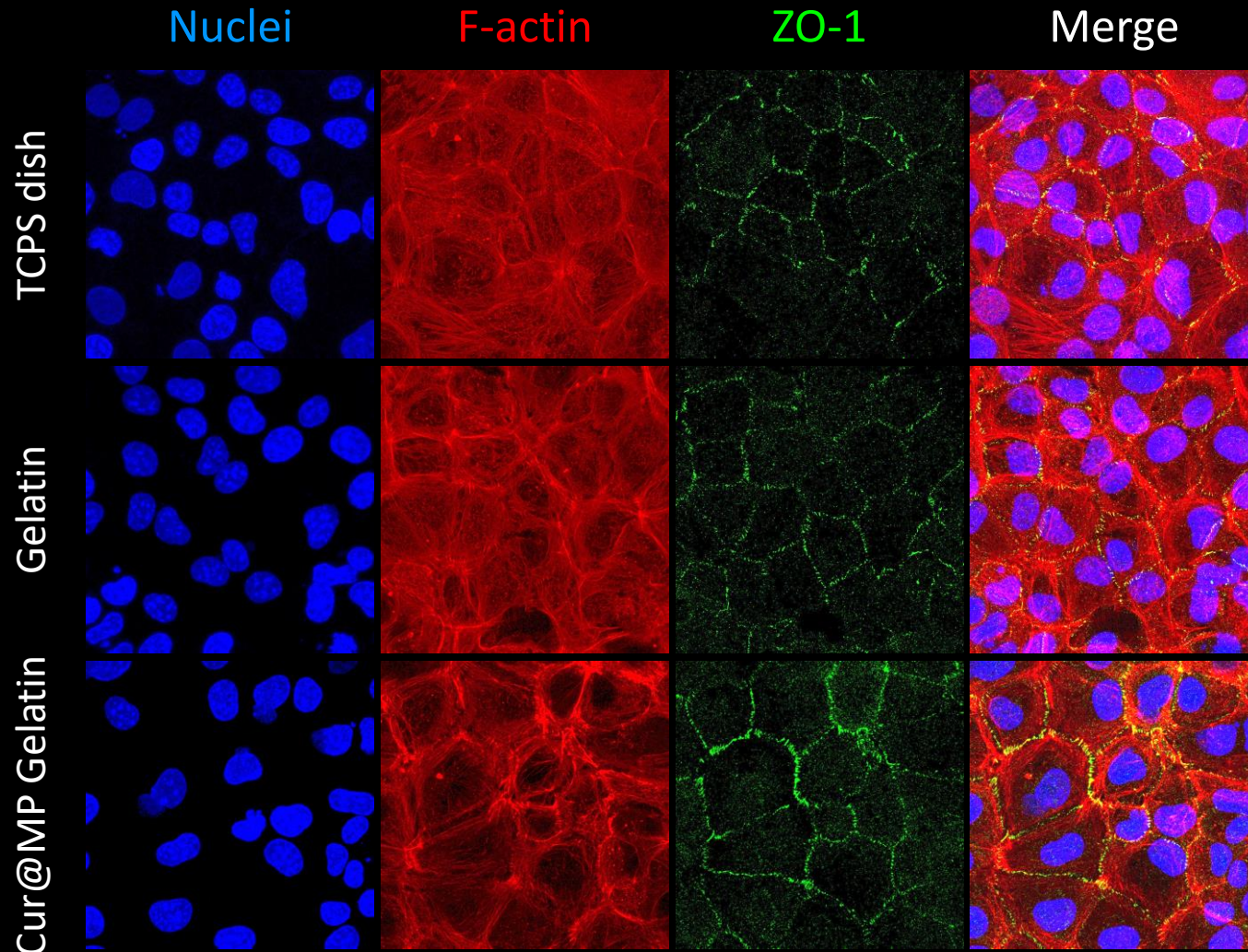
Scaffolds for Regenerating Corneal Endothelium

Scaffold Fabrication



Scaffolds for Regenerating Corneal Endothelium

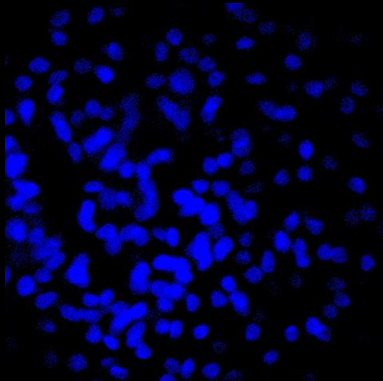
Human corneal endothelial cell line (B4G12 cells)



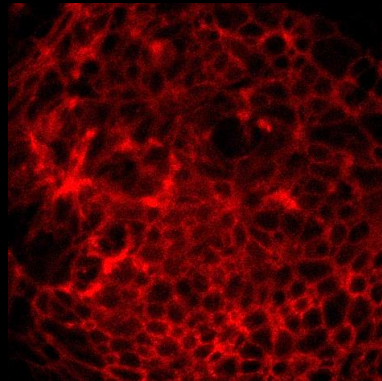
Scaffolds for Regenerating Corneal Endothelium

Primary rabbit corneal endothelial cells

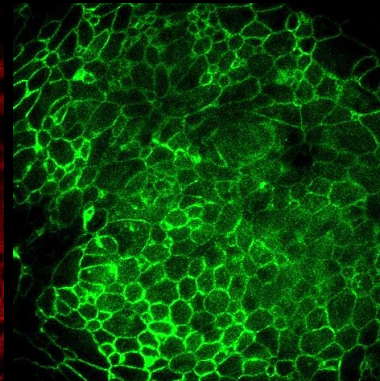
Nuclei



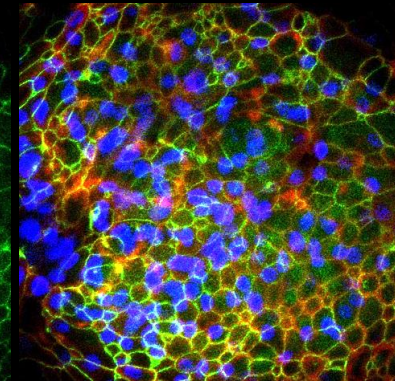
F-actin



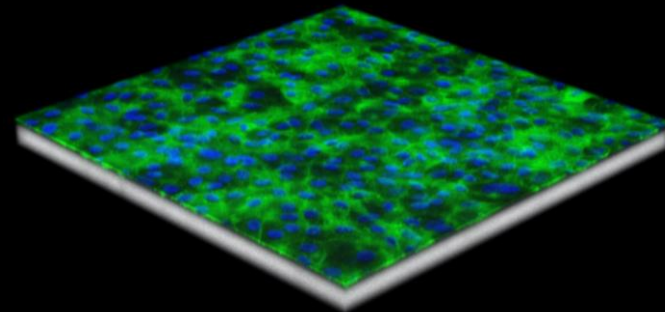
ZO-1



Merge



Nuclei ZO-1 Gelatin

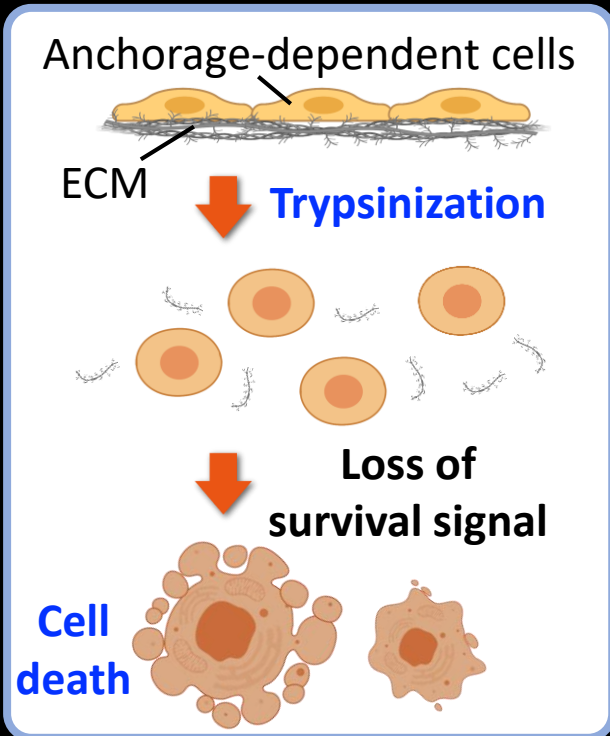


Summary III

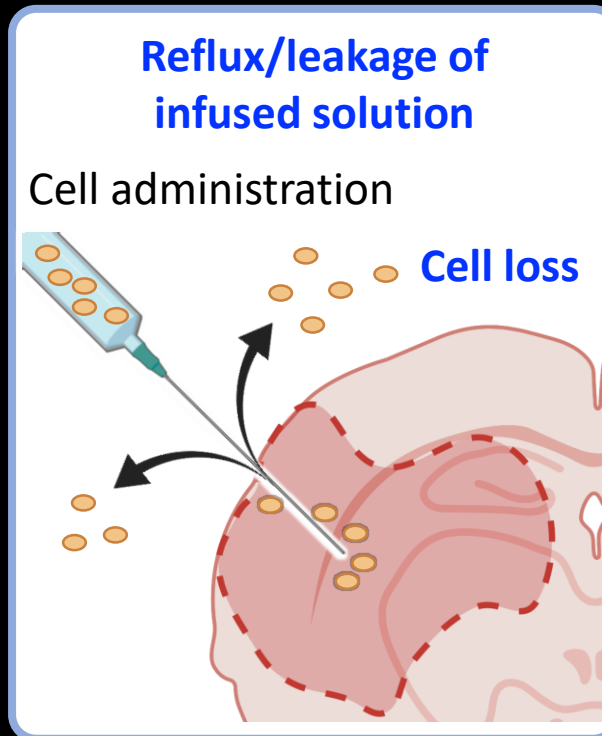
- Engineer a suitable environment for delivered cells
- Improve the **viability** of cells under harsh condition
- Enhance cellular **functionality**

1. 3D Cell Spheroid
2. Functional Biomaterials

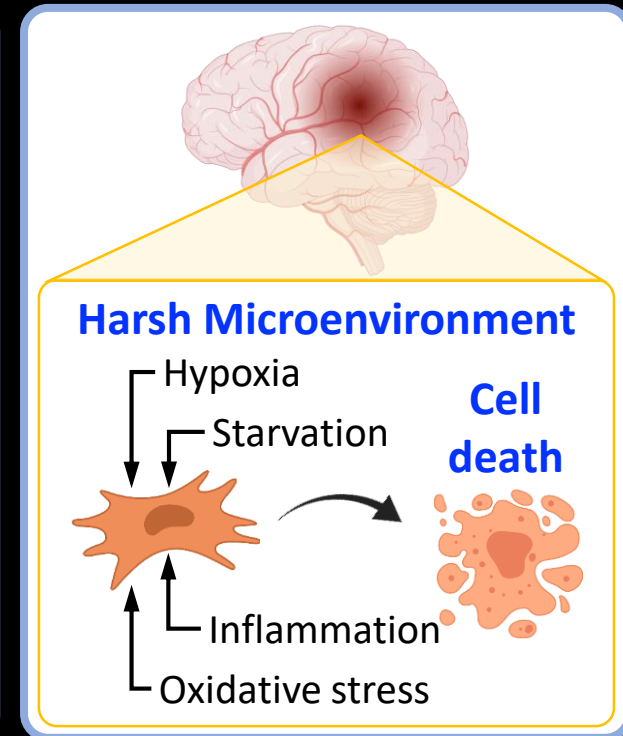
Anoikis



Cell dispersal



Hostile target tissue



Acknowledgement



Ministry of Science and Technology (MOST)

National Tsing Hua University

Linkou Chang Gung Memorial Hospital



Thanks for Your Attention