Out-of-body SANS Research

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The Ex-vivo Human Translaminar Autonomous System To Study Space Associated Neuro-ocular Syndrome Pathogenesis

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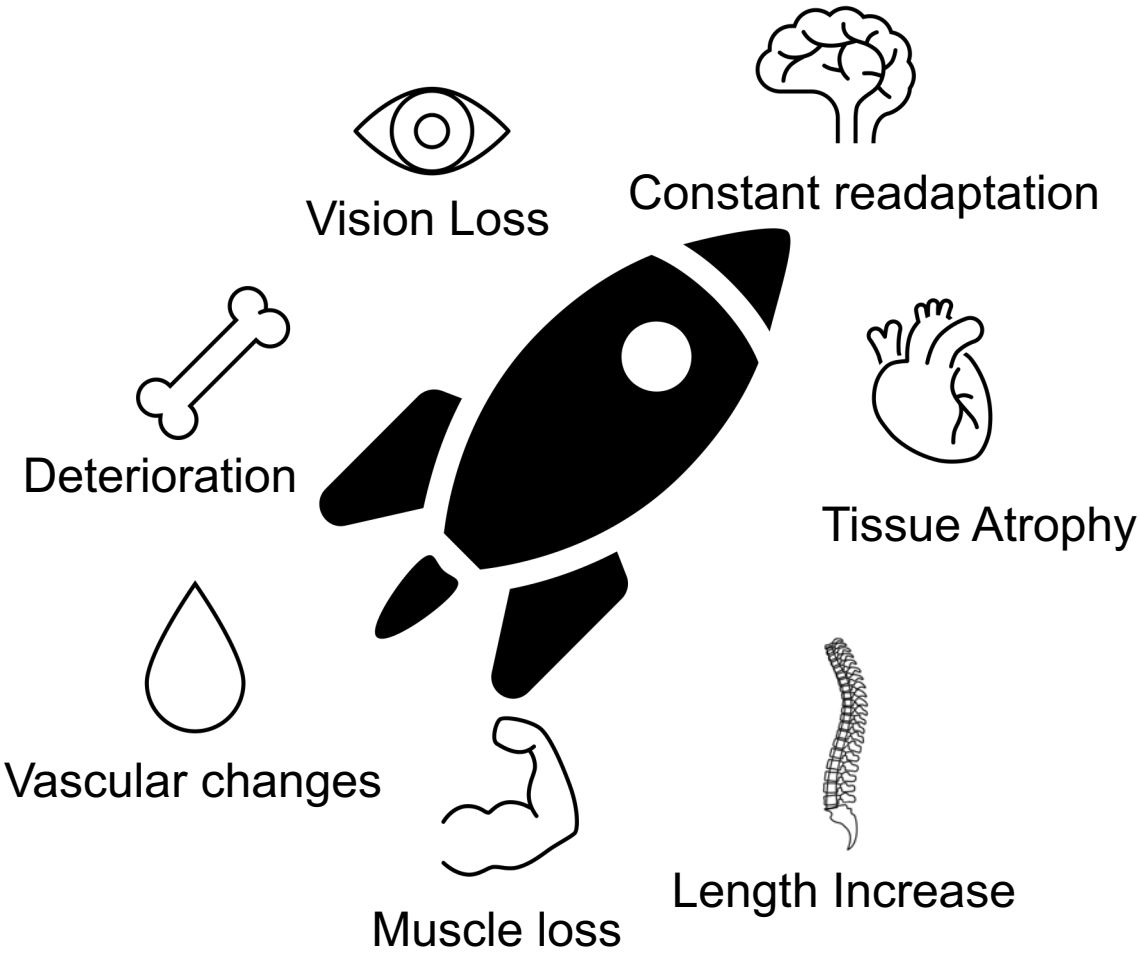
What happens to our bodies in SPACE?

- Cosmic radiation
- Microgravity
- Stress
- Insomnia
- Increased pressure
- Vascular pressure
- Tissue Atrophy

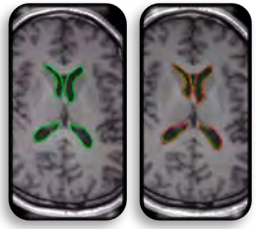


https://www.science.org.au/curious/bodies-in-space

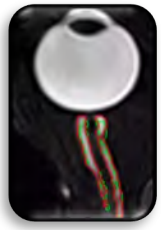
Spaceflight Associated Neuro-Ocular Syndrome

No gravity

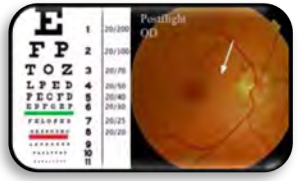
Increased intracranial CSF



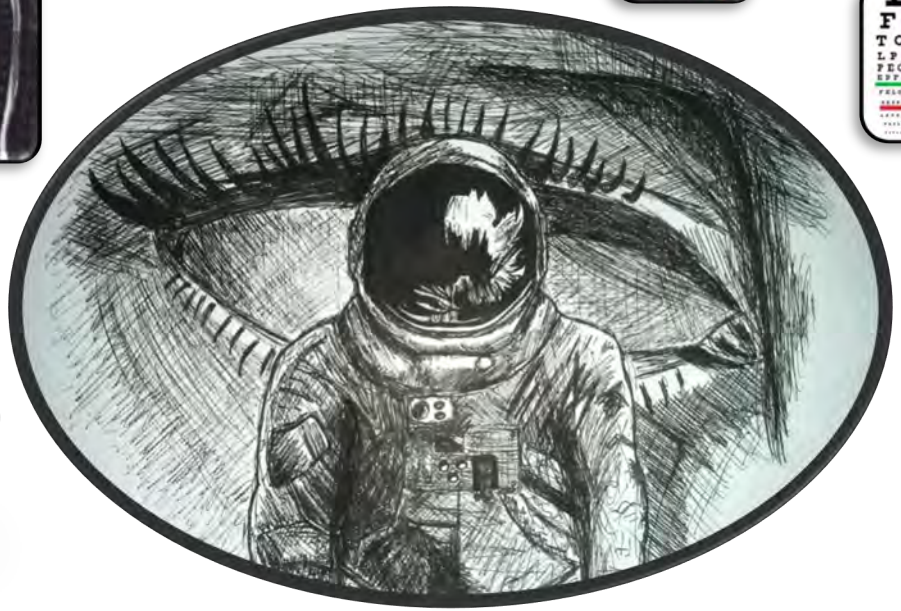
Increased orbital CSF



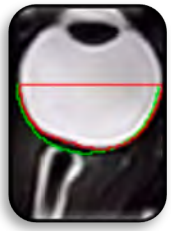
Hyperopic shift and optic disc edema



Long duration spaceflight

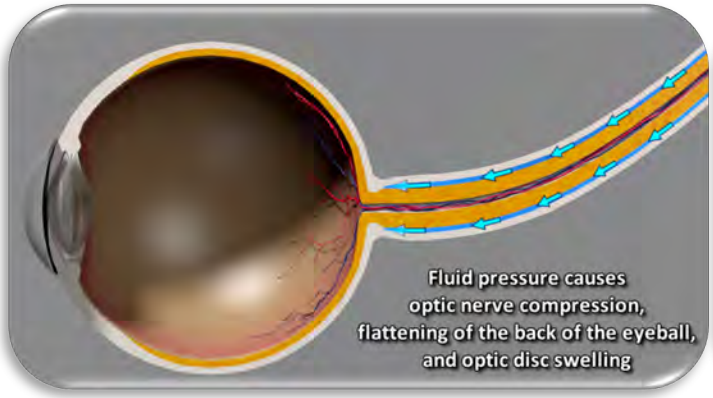


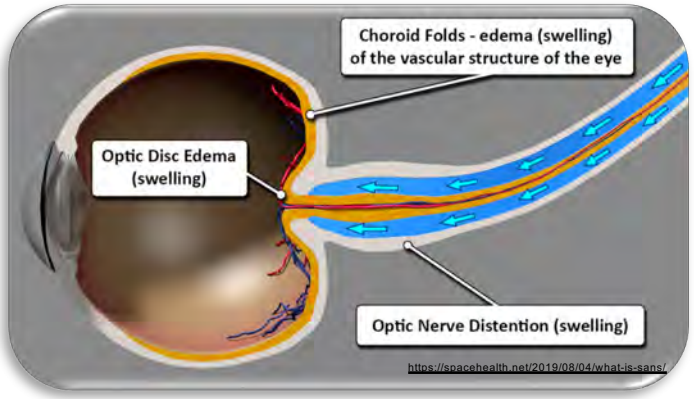
Globe deformations: Flattening and protrusion

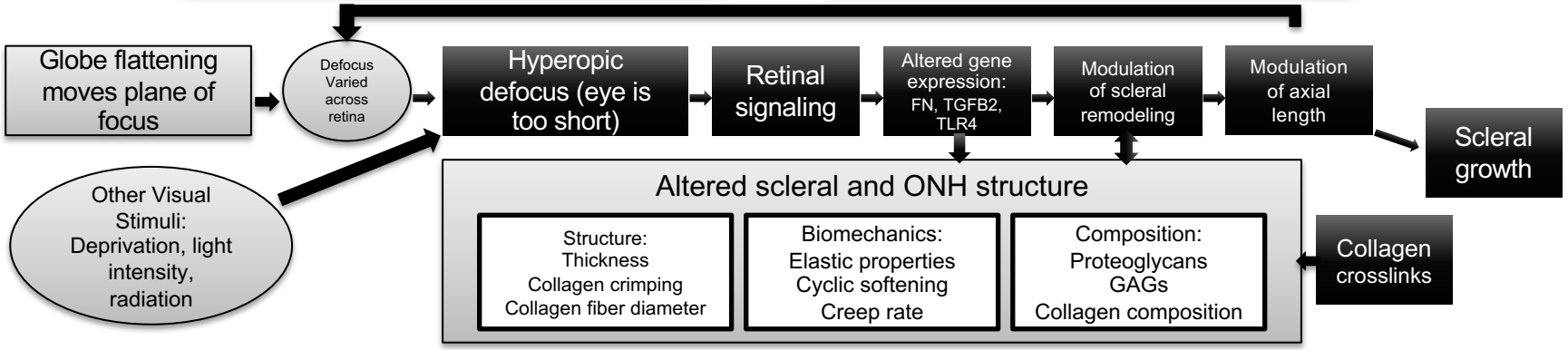


https://newatlas.com/blurry-vision-cause-astronauts-spinal-fluid/46667/#p437864

SANS Pathogenesis







Stockslager et al. 2016

NASA Risks

1. SANS is considered an unexplained major risk factor
2. Understand the pathogenesis associated with SANS:
   - Optic nerve head
   - Optic nerve
   - Retinal ganglion cells

NASA Gaps

SANS-102: Determine the relationship between the fluid-shifts induced ocular changes and fluid shifts in the CNS, including whether elevated intracranial pressure or brain edema play a role.

1. What are the etiological mechanisms and contributing risk factors for ocular structural and functional changes seen in-flight and postflight?

2. Are there any ground-based analogs and/or models can simulate Space Associated Neuro-ocular Syndrome?

Potential Mechanisms of SANS

- Increased intracranial pressure
- Translaminar pressure gradient
- Cephalad fluid shift with volume increase
- Altered glymphatic drainage
- Intracerebral volume and cerebral edema alterations
- Orbital and cerebral arterial or venous drainage defects
- One carbon pathway metabolism alterations
- Choroidal volume expansion
- Hypercapnia related volume and pressure disturbances

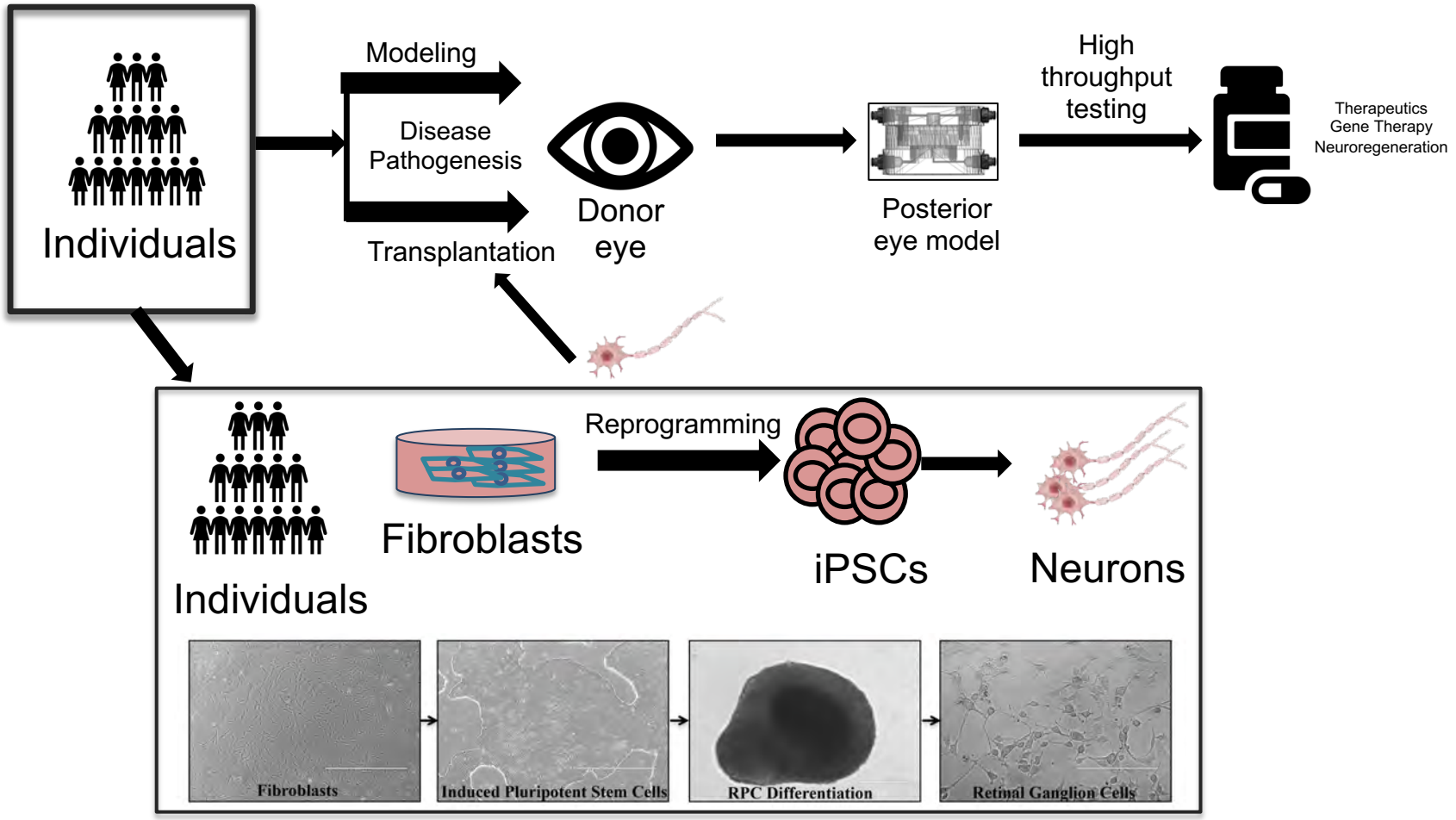
Exact etiology/pathophysiology not known

Changes in pressure gradients within the eye

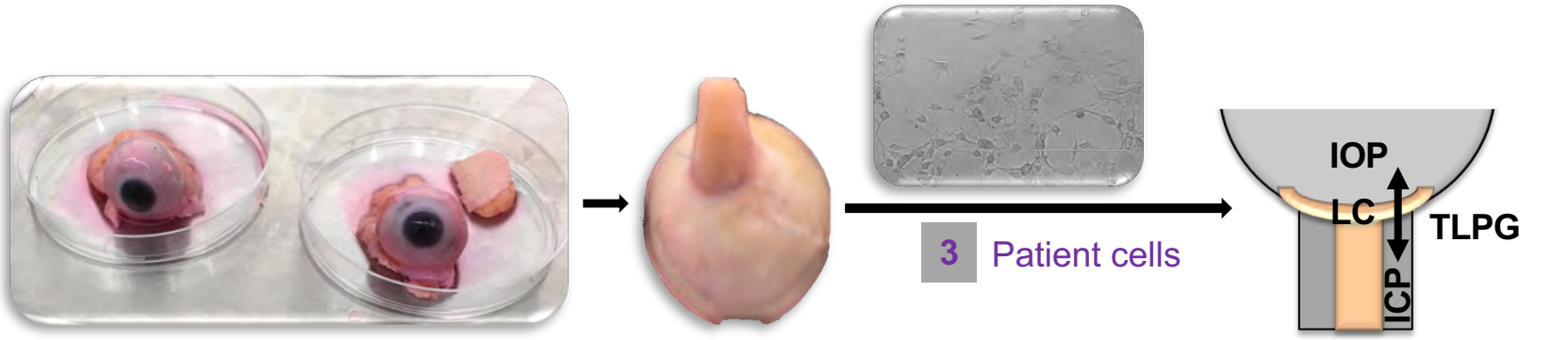
NASA Path to Risk Reduction

1. How do we preclinically in human tissue evaluate:
   - Pathogenesis
   - Therapeutics
2. How do we identify variability in SANS between astronauts?
3. Is it possible to perform precision medicine?

Overview

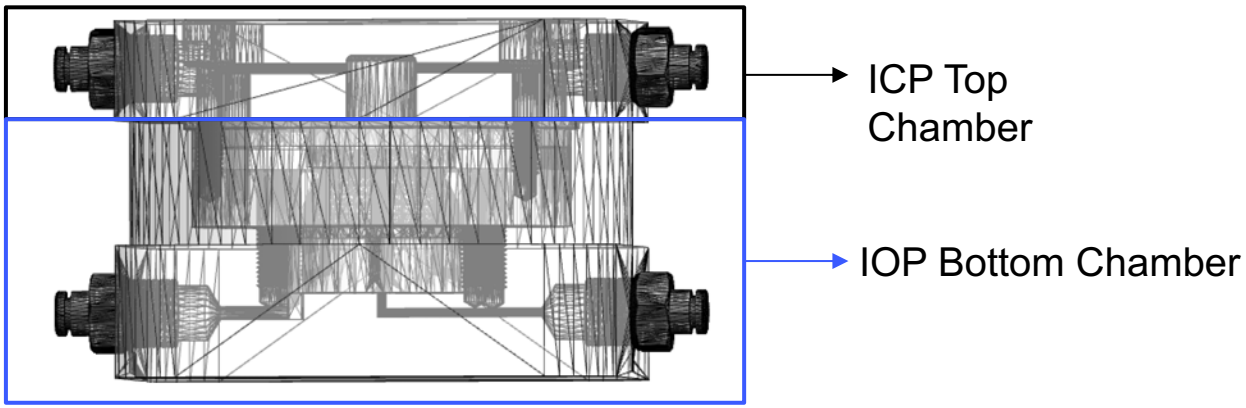

Individuals
Modeling
Disease Pathogenesis
Transplantation
Donor eye
Posterior eye model
High throughput testing
Therapeutics
Gene Therapy
Neuroregeneration
Individuals
Fibroblasts
Reprogramming
iPSCs
Neurons
Fibroblasts
Induced Pluripotent Stem Cells
RPC Differentiation
Retinal Ganglion Cells

Model to study TLPG ex-vivo

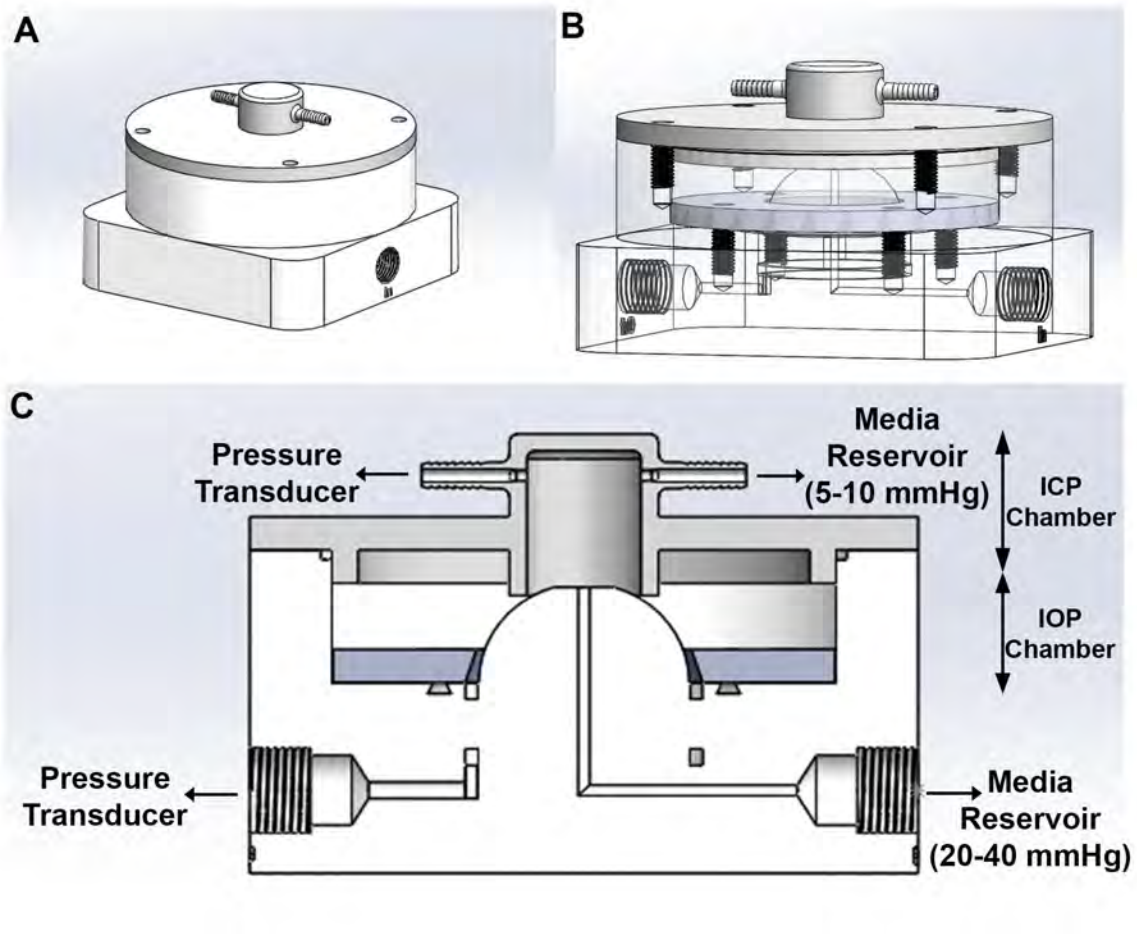


Translaminar Autonomous System

A novel ex-vivo human ocular model to test drug therapies, compounds, and transplantation strategies preclinically in a cost-effective and non-invasive manner.



Translaminar Autonomous System



Donor human eye

Viable for 30 days

Sterile

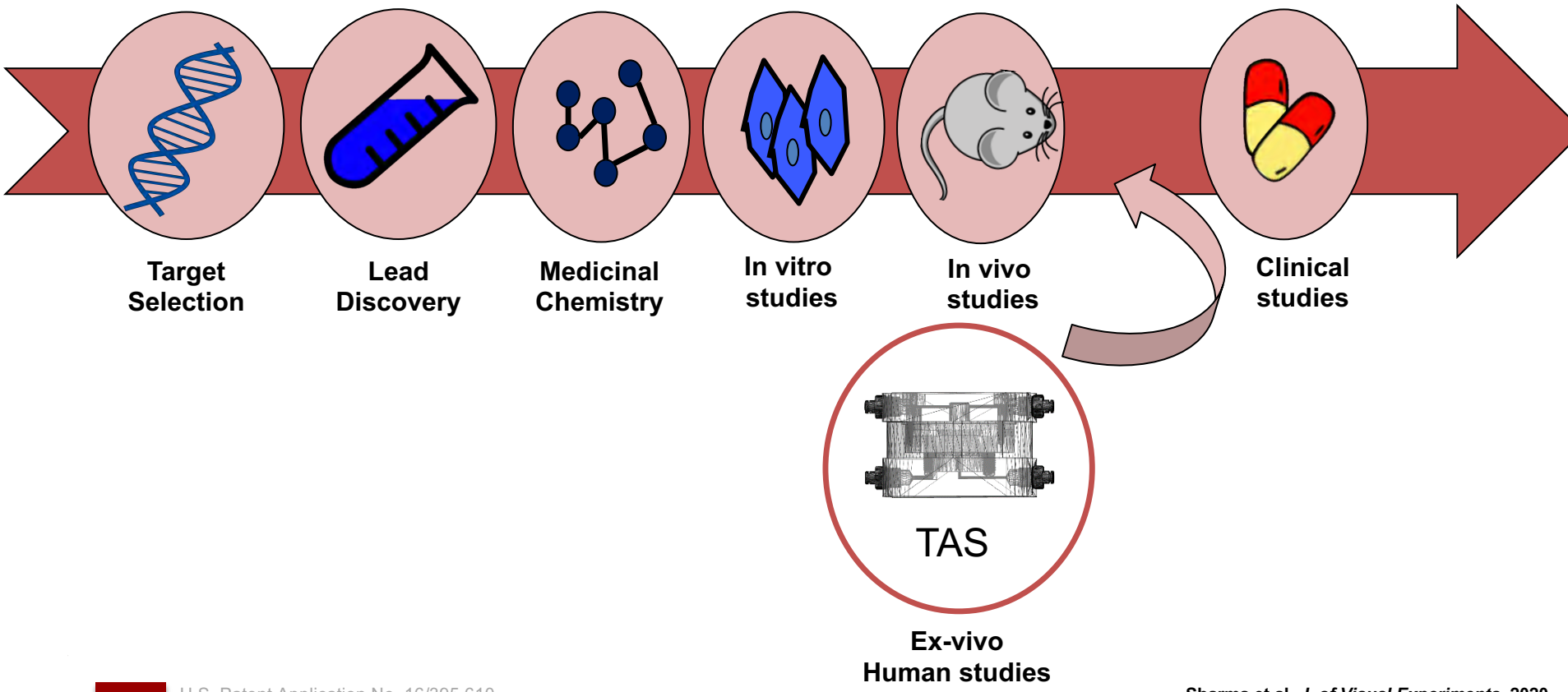
Internal/External pressure testing

Infusion of medium
- Therapies
- Drug targets
- Biomarkers

U.S. Patent Application No. 16/395,610

Sharma et al. *J. of Visual Experiments*. 2020

Opportunity of TAS Model



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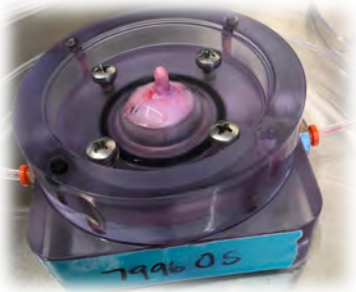
Sharma et al. *J. of Visual Experiments*. 2020

Validating the Model

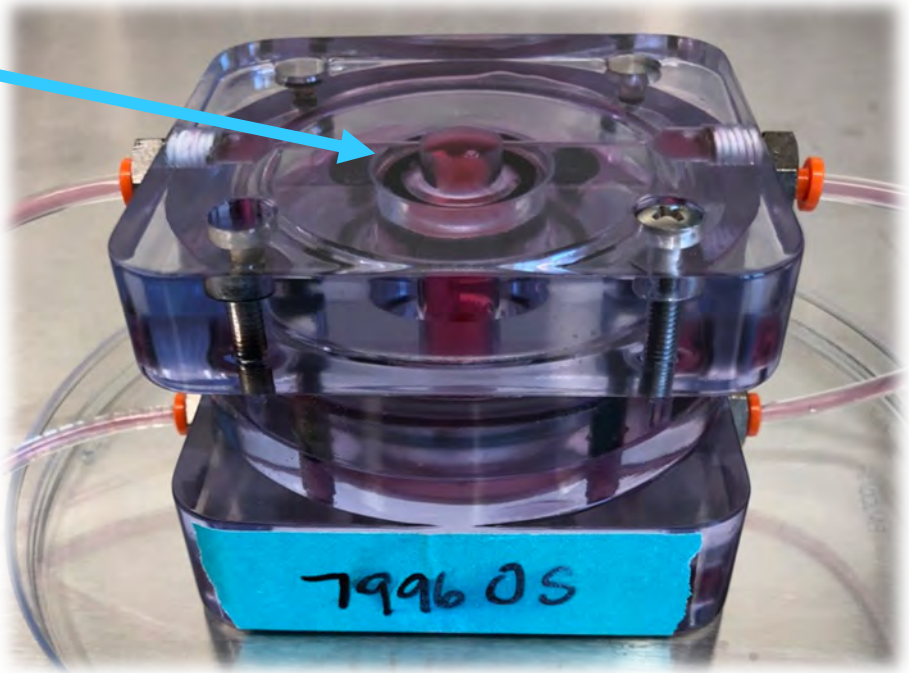
Translaminar Autonomous System

Top Chamber



Bottom Chamber

Independent
Sealed
Pressurized



Model Mechanics

U.S. Patent Application No. 16/395,610

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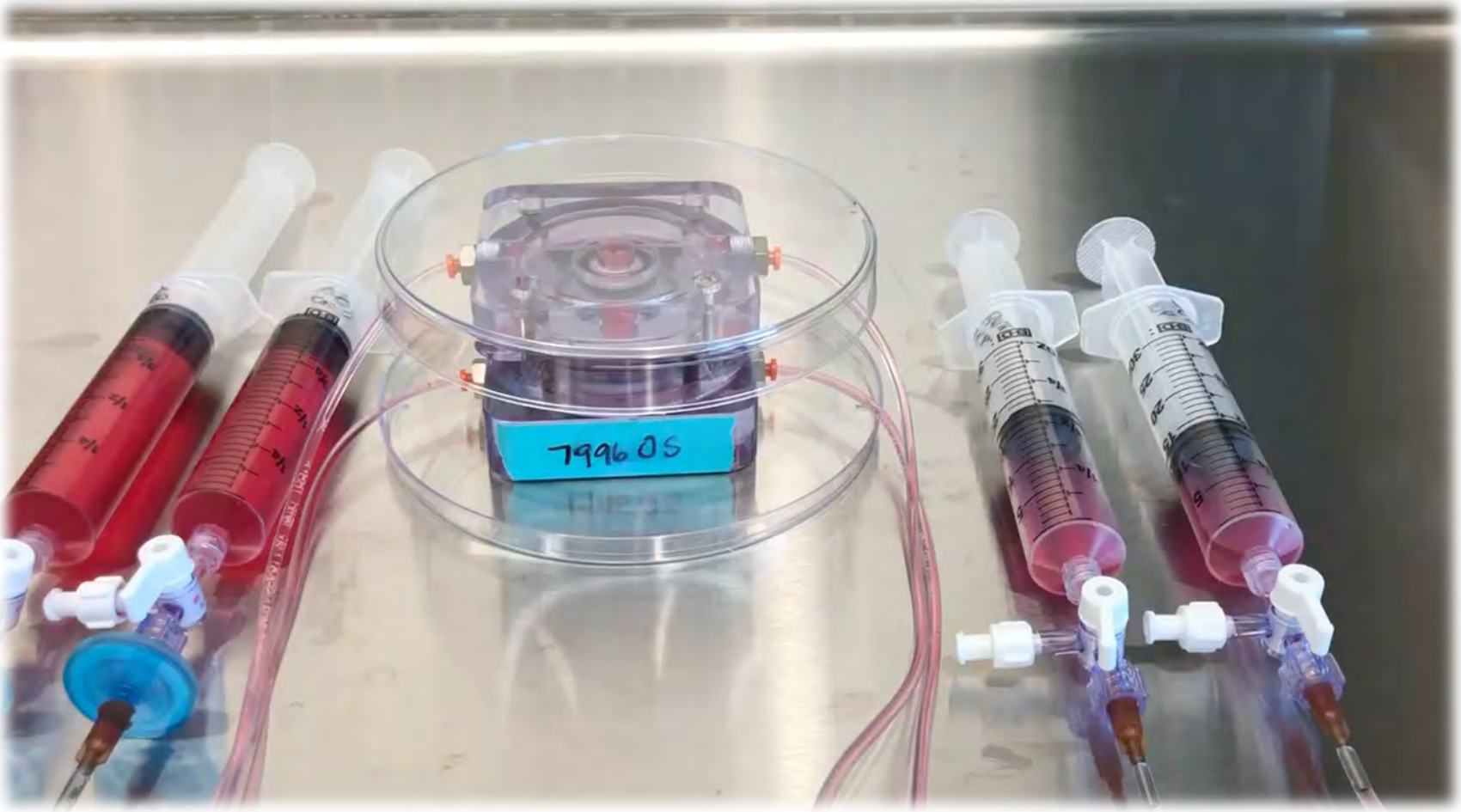
Model Mechanics



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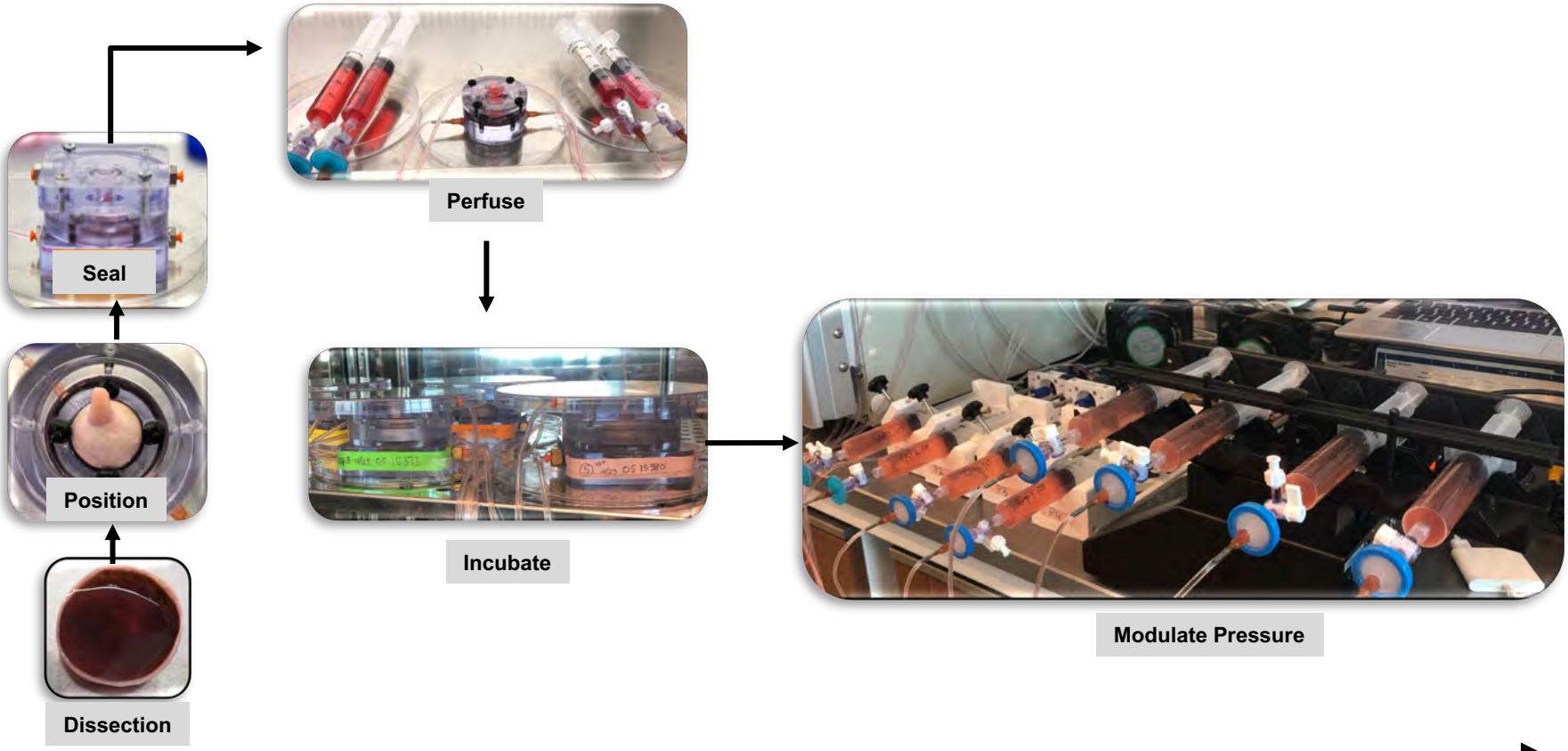
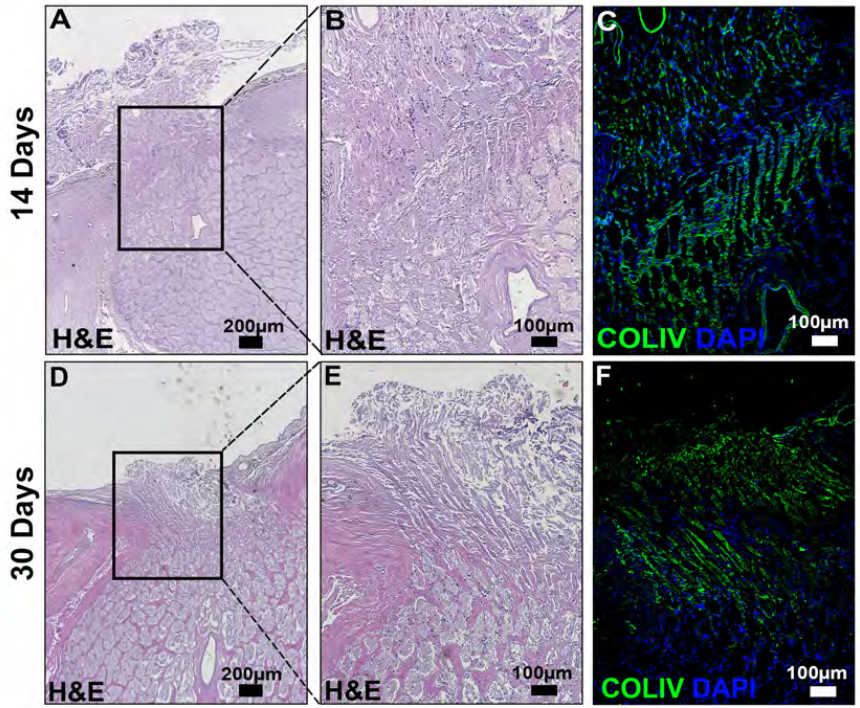
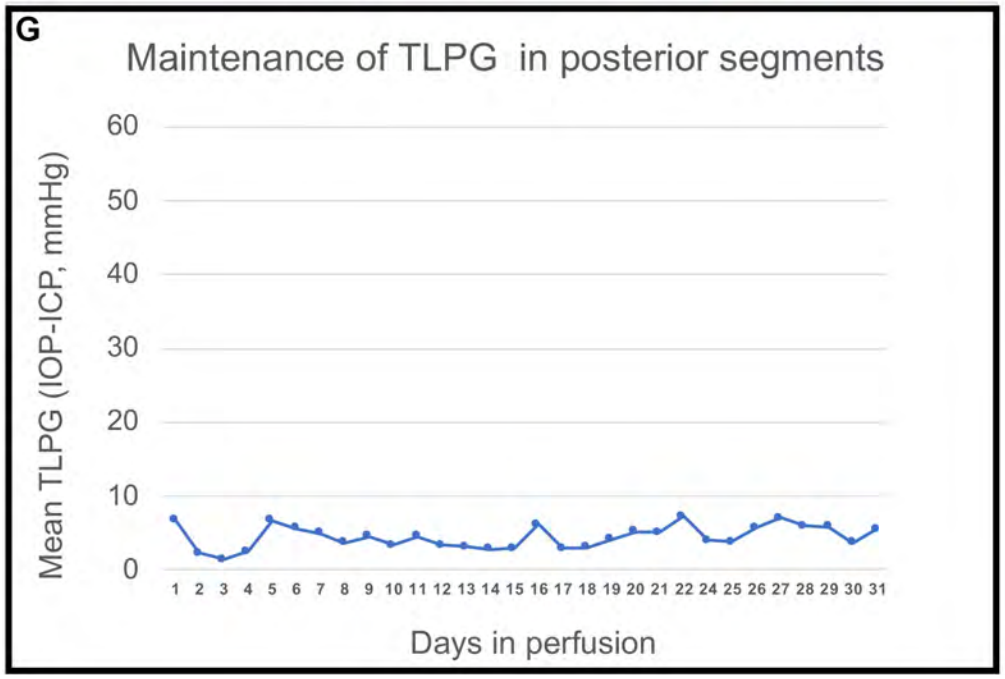
Methods


Perfuse
Seal
Position
Incubate
Modulate Pressure
Dissection
5 Hours

Successful Culture Of Human Eyes In System

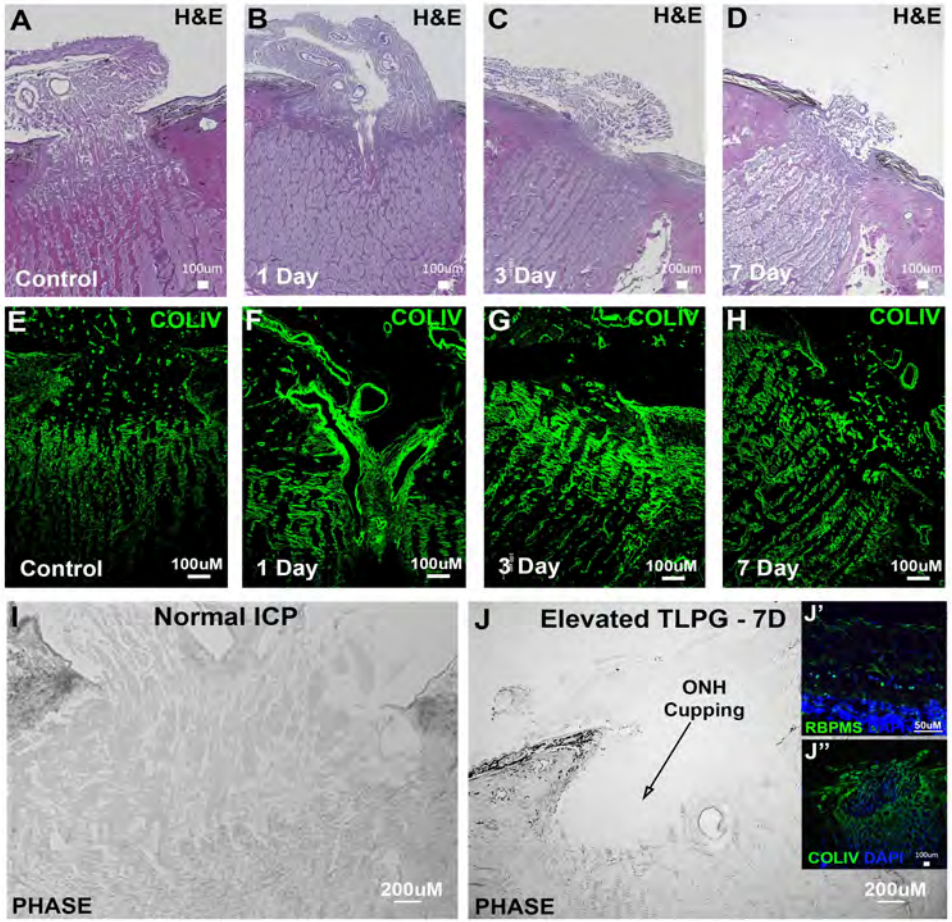


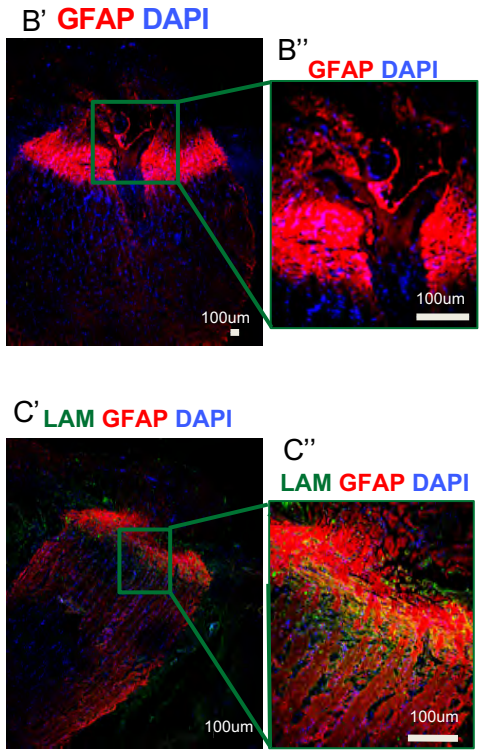
Health and integrity of tissue maintained



Constant maintenance of 2-5mmHg of TLPG

Reorganization and Cupping of ONH in TAS Model





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Translaminar Autonomous System

1. The model is the first of its kind

2. Regulates IOP and ICP autonomously

3. An *ex vivo* paradigm to study SANS

Sharma et al. ***J. of Visual Experiments*. 2020**

U.S. Patent Application No. 16/395,610

Part 1- TRISH funded Disruption

Connection to TRISH

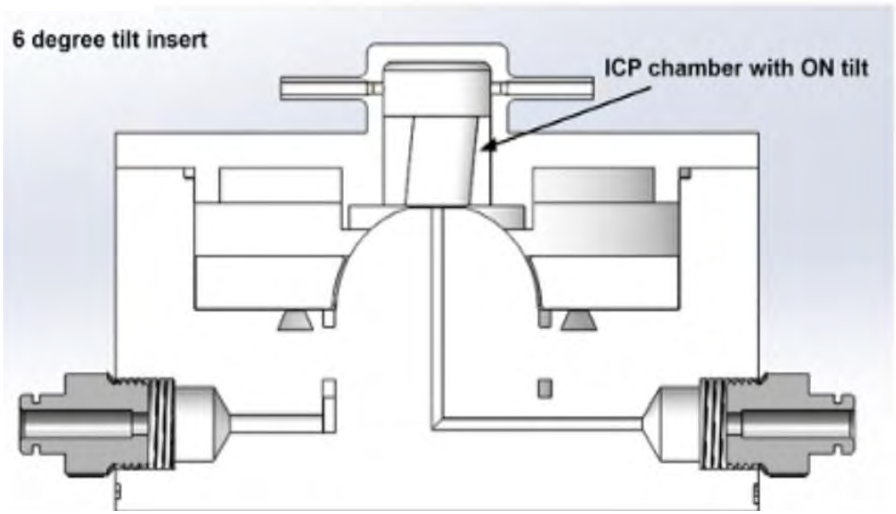
1. First ground-based analog to ex-vivo mimic mildly elevated ICP in human eyes
2. Is TAS a suitable ground-based analog to study the SANS phenomenon?
3. TRISH- define parameters and feasibility of using our model to understand SANS pathogenesis
   - A high-risk project
   - Validating a novel preclinical human translaminar model
   - Prototype development

Utilization of TAS Model for SANS

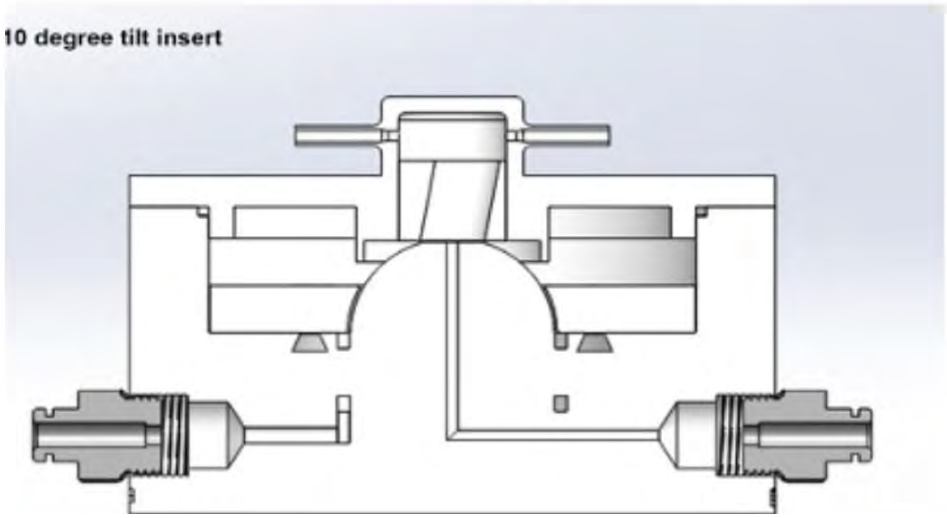
1. Can we characterize the pathological changes occurring within the human posterior eyecup due to elevated IOP/ICP or ON tortuosity?

2. Can we determine the transport and functional capacity of RGCs within the human posterior eyecup after elevated IOP/ICP or ON tortuosity?

Variations to model for SANS



Diagrammatic view of 6° tilt

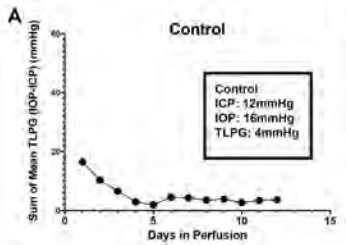


Diagrammatic view of 10° tilt

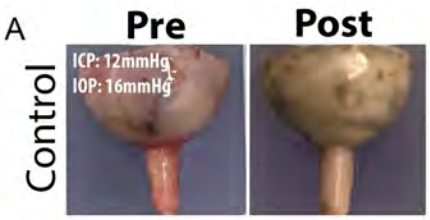
Experimental Paradigms Tested

Control	Group 1	Group 2	Group 3	Group 4	Group 5
ICP: 12mmHg IOP: 16mmHg TLPG: 4mmHg	ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	ICP: 12mmHg IOP: 21mmHg(7d) 14mmHg TLPG: 2-9mmHg	ICP: 21mmHg IOP: 15mmHg TLPG: 6mmHg	6 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	10 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg

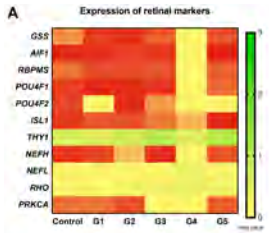
Major goals for SANS Validation



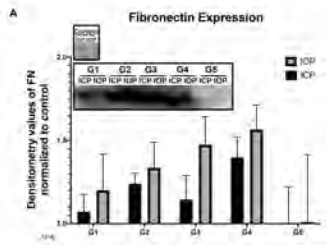
Validation of pressure



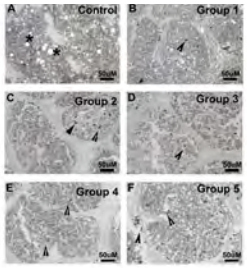
Posterior globe changes



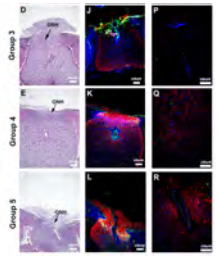
Identification of markers



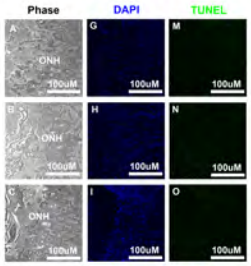
Secreted ECM proteins



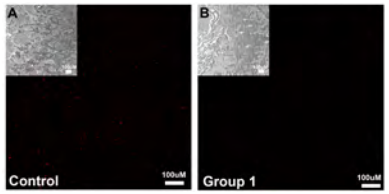
Degeneration of axons



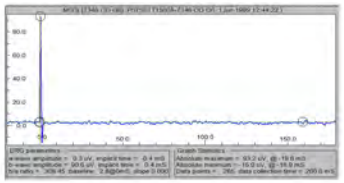
Reorganization and gliosis



Apoptosis

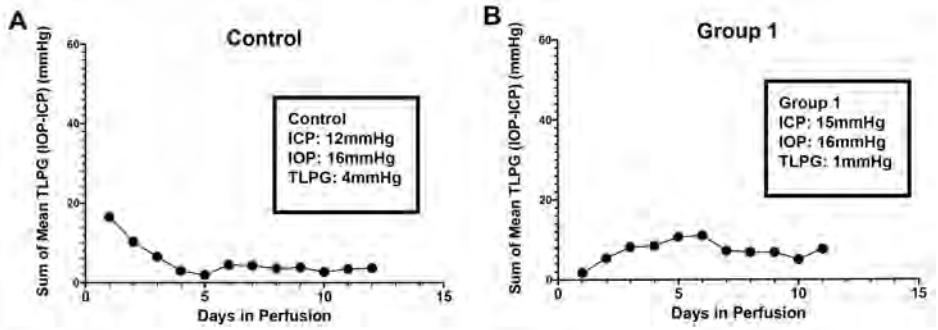


Anterograde transport

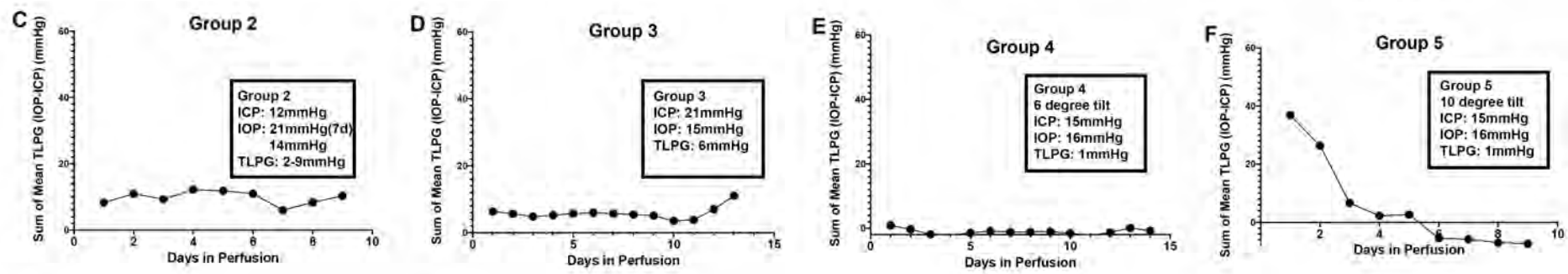


Electroretinogram analysis

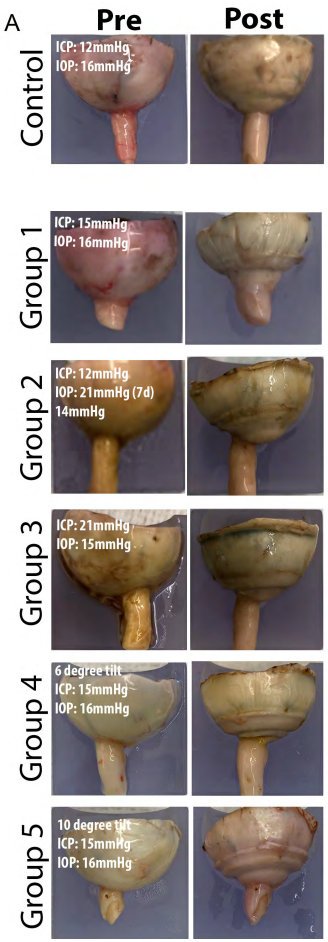
Maintenance of translaminar pressure gradients

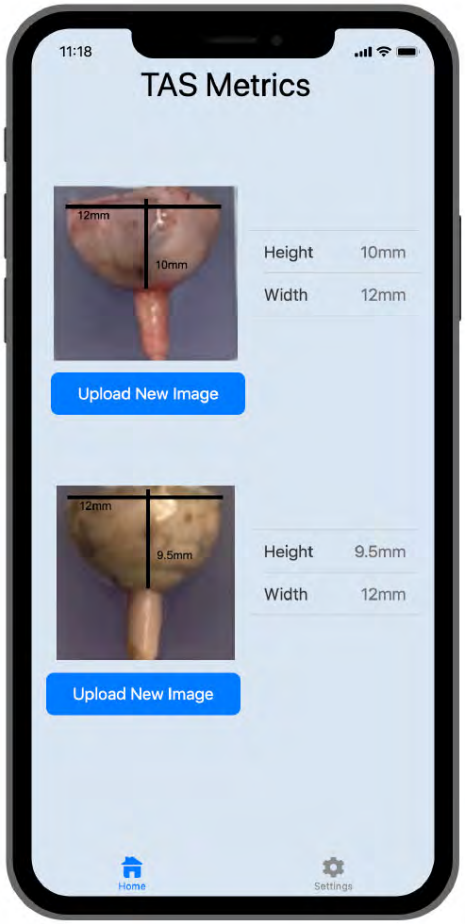


Graphical representation of sum of mean TLPG (Δ in mmHg of IOP-ICP every 24 hours)



Analysis of posterior human globes pre- and post-culture in the TAS model





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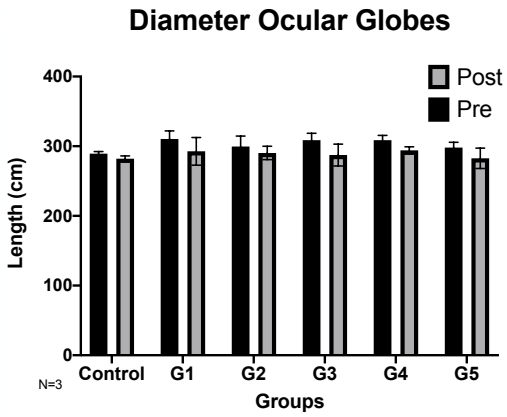
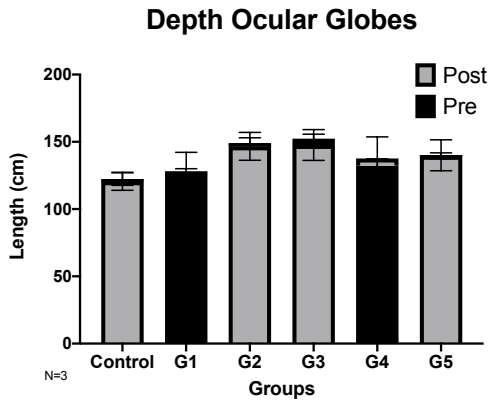
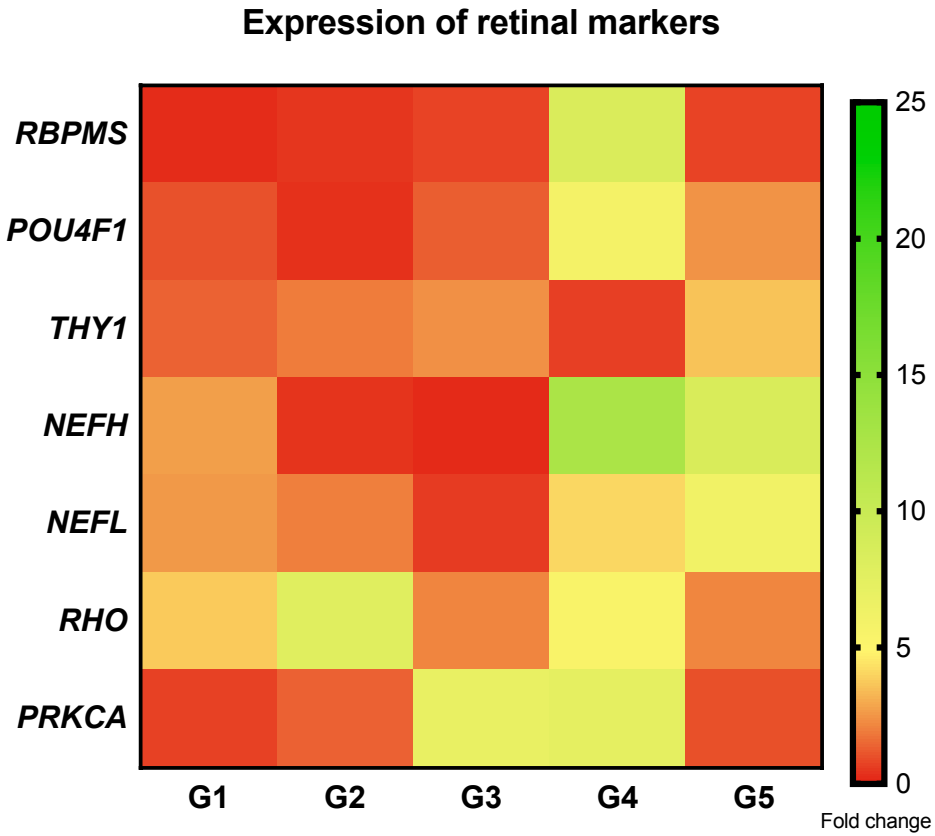
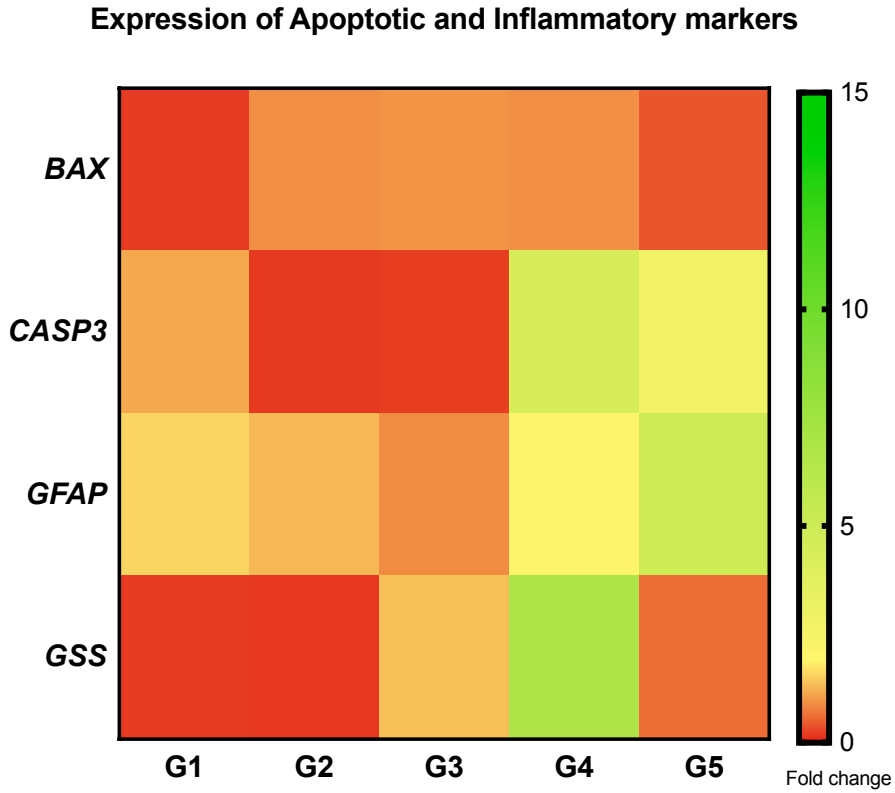




Expression of retinal markers with increase in apoptosis and inflammation in experimental groups post culture





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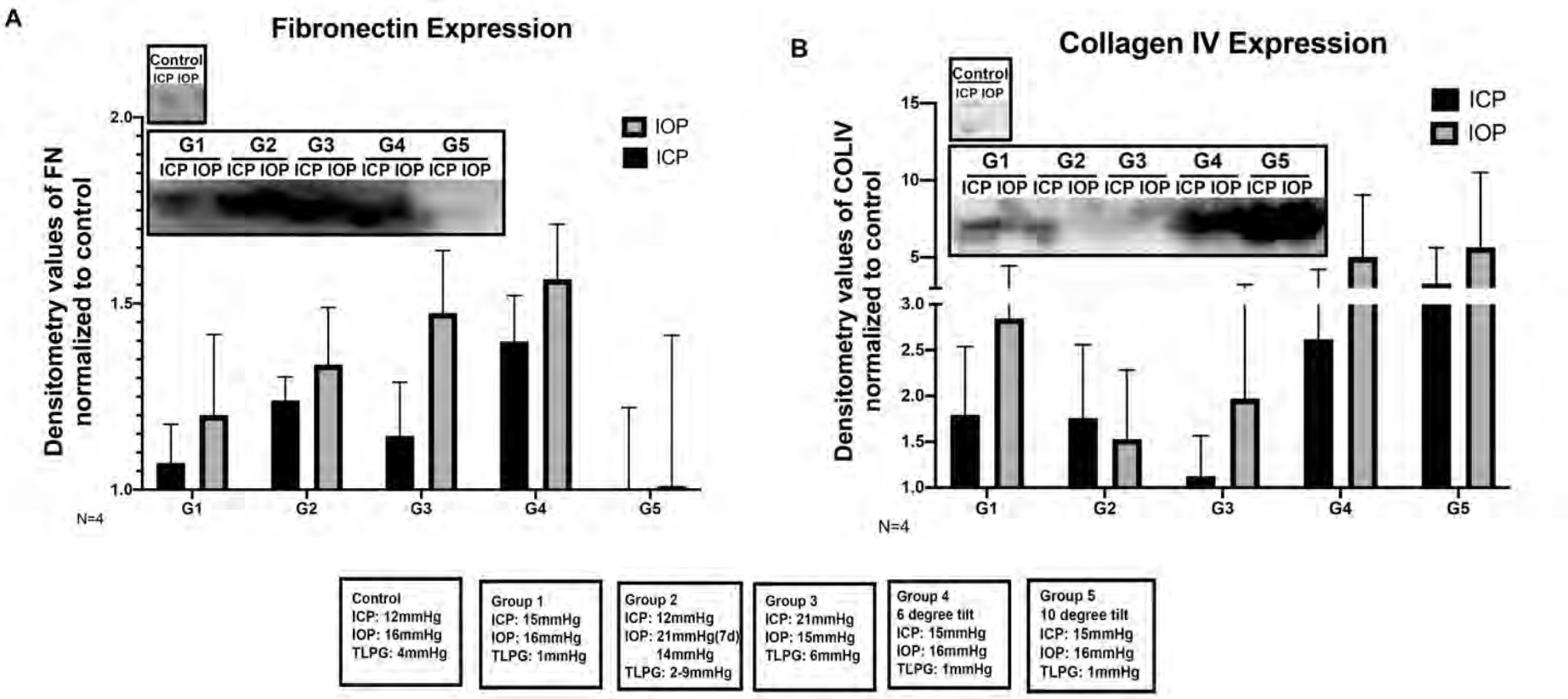
ICP: 12mmHg IOP: 16mmHg TLPG: 4mmHg	ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	ICP: 12mmHg IOP: 21mmHg(7d) 14mmHg TLPG: 2-9mmHg	ICP: 21mmHg IOP: 15mmHg TLPG: 6mmHg	6 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	10 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg

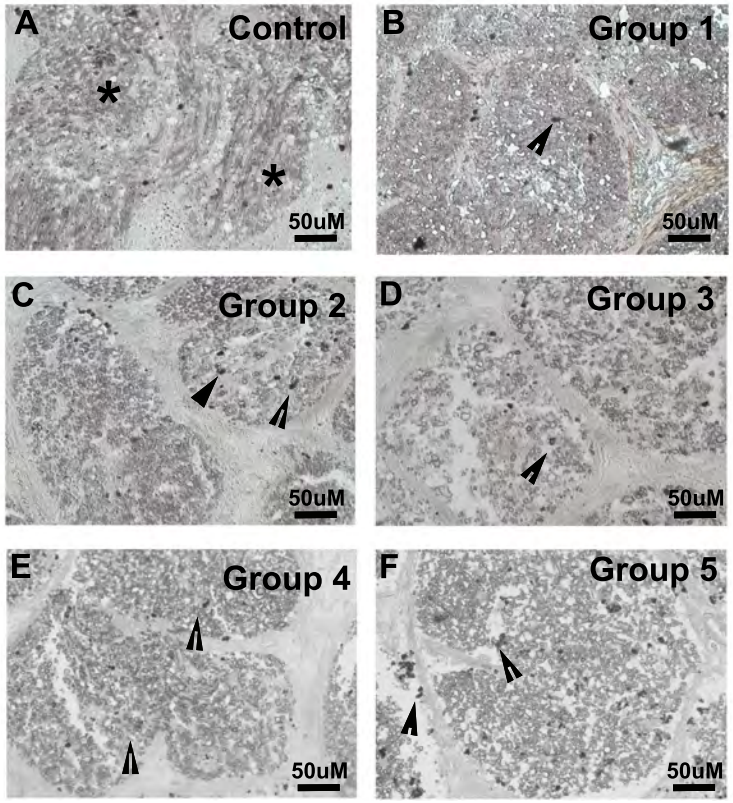
Increased extracellular matrix secretion observed in ICP and IOP chamber conditioned medium of experimental groups

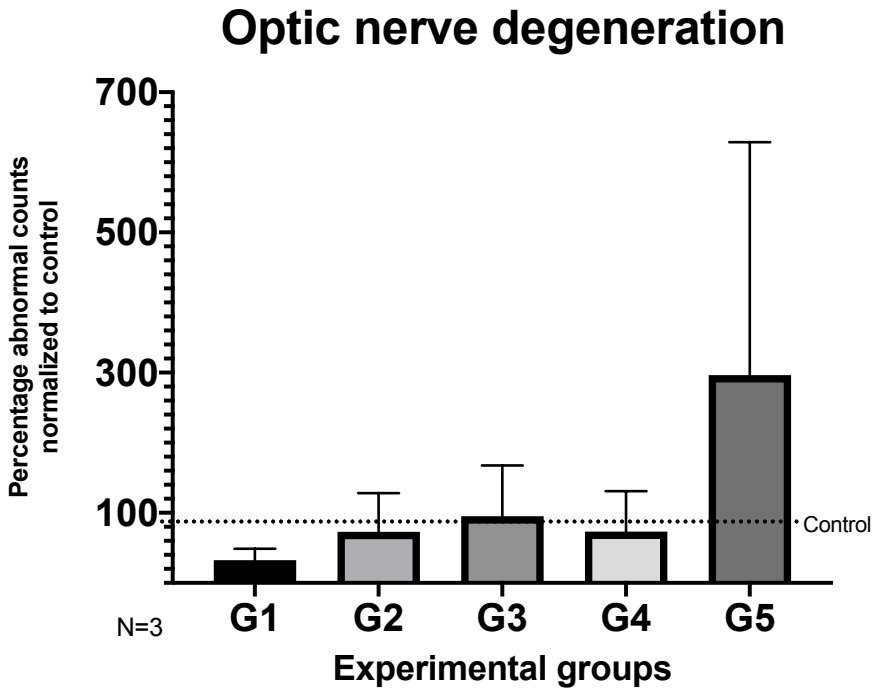


Control	Group 1	Group 2	Group 3	Group 4	Group 5
ICP: 12mmHg IOP: 16mmHg TLPG: 4mmHg	ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	ICP: 12mmHg IOP: 21mmHg(7d) 14mmHg TLPG: 2-9mmHg	ICP: 21mmHg IOP: 15mmHg TLPG: 6mmHg	6 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	10 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg

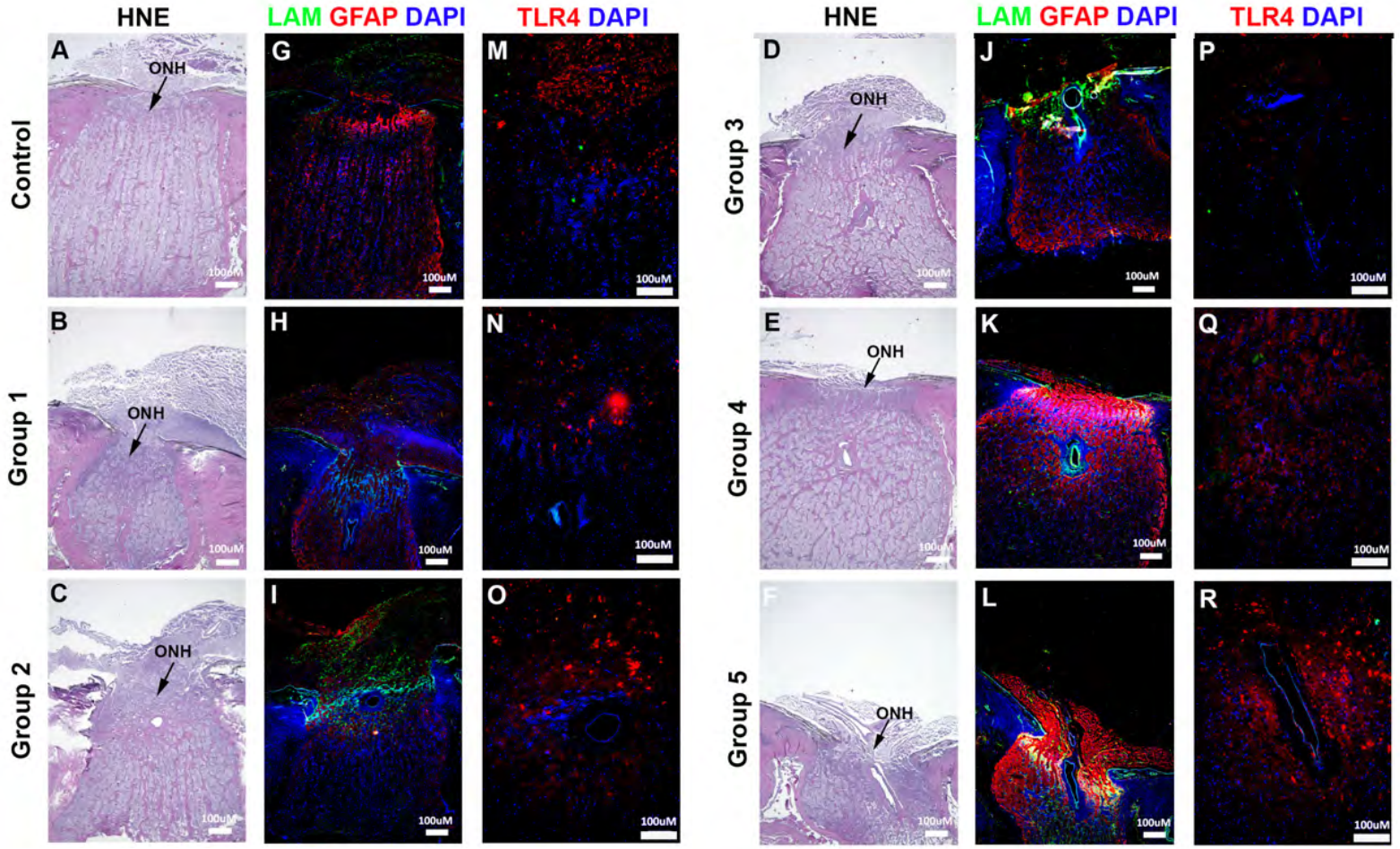
Increased degeneration of optic nerve axons observed under experimental conditions

Control	Group 1	Group 2	Group 3	Group 4	Group 5
ICP: 12mmHg IOP: 16mmHg TLPG: 4mmHg	ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	ICP: 12mmHg IOP: 21mmHg(7d) 14mmHg TLPG: 2-9mmHg	ICP: 21mmHg IOP: 15mmHg TLPG: 6mmHg	6 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	10 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg

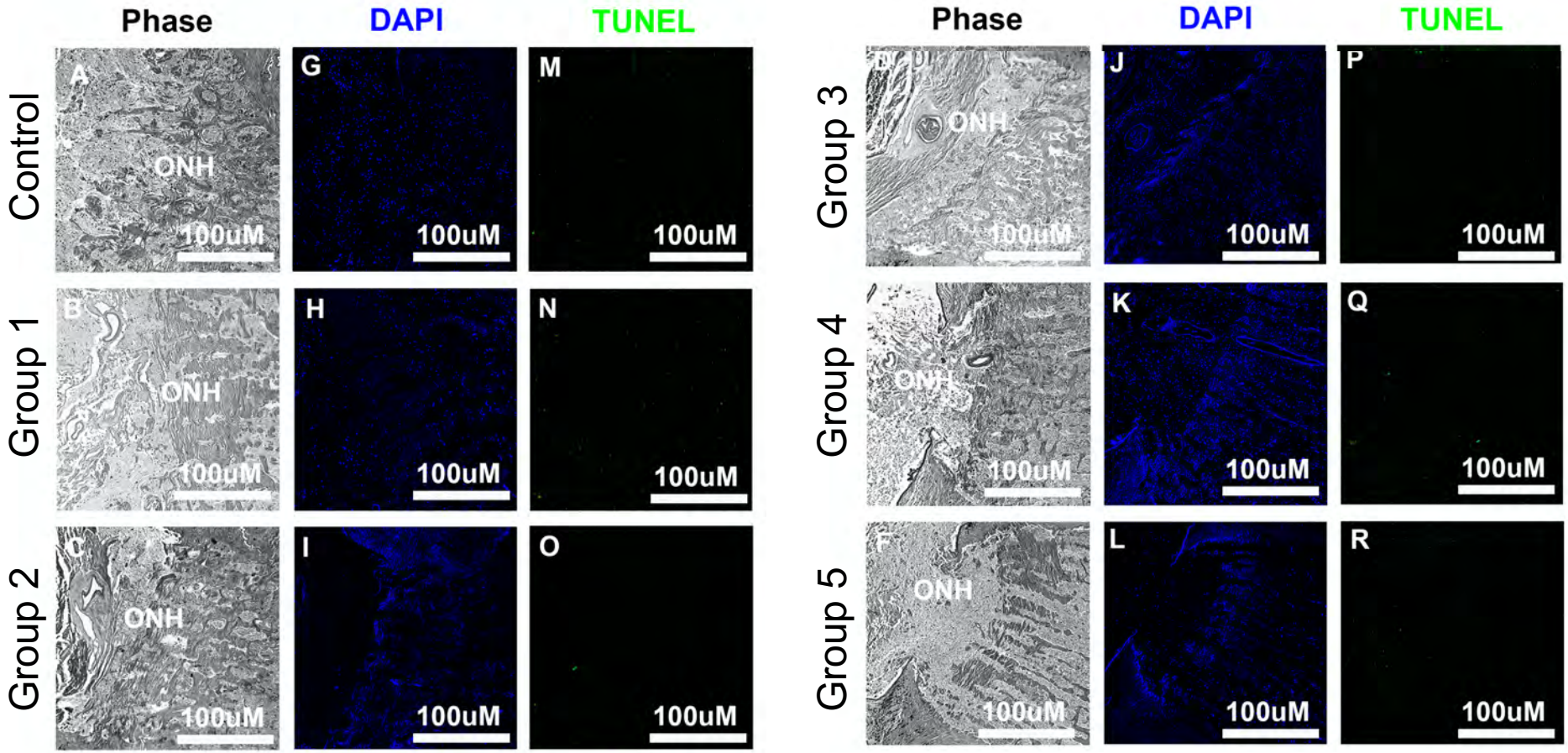




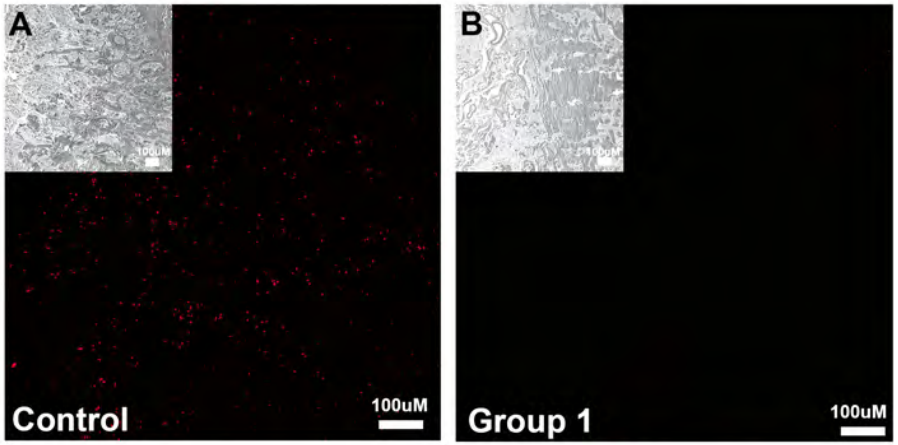
Morphological restructuring of the optic nerve head after simulation of SANS conditions in the TAS model



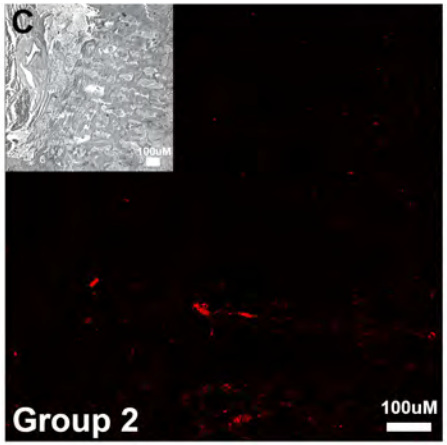
Increased TUNEL positive cells identified within the optic nerve head of experimental groups

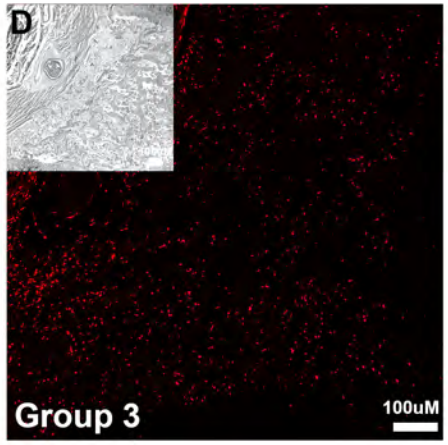


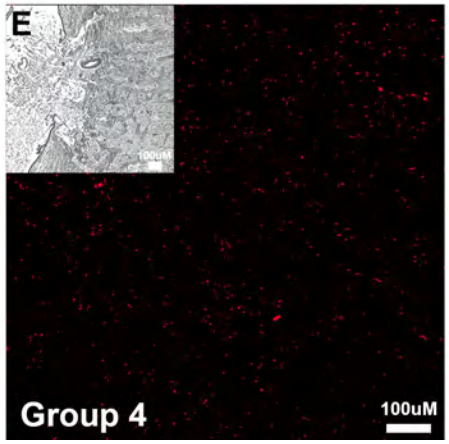
Minimal expression of Cholera-Toxin B within the optic nerve head cross-sections

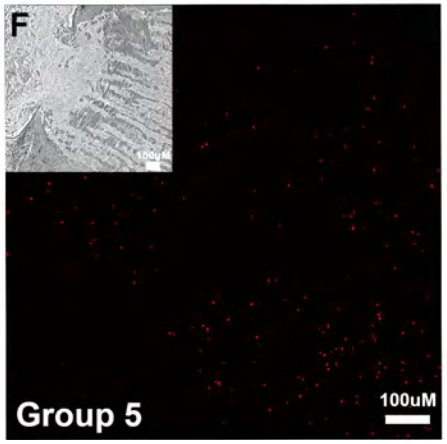


Control	Group 1	Group 2	Group 3	Group 4	Group 5
ICP: 12mmHg IOP: 16mmHg TLPG: 4mmHg	ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	ICP: 12mmHg IOP: 21mmHg(7d) 14mmHg TLPG: 2-9mmHg	ICP: 21mmHg IOP: 15mmHg TLPG: 6mmHg	6 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg	10 degree tilt ICP: 15mmHg IOP: 16mmHg TLPG: 1mmHg

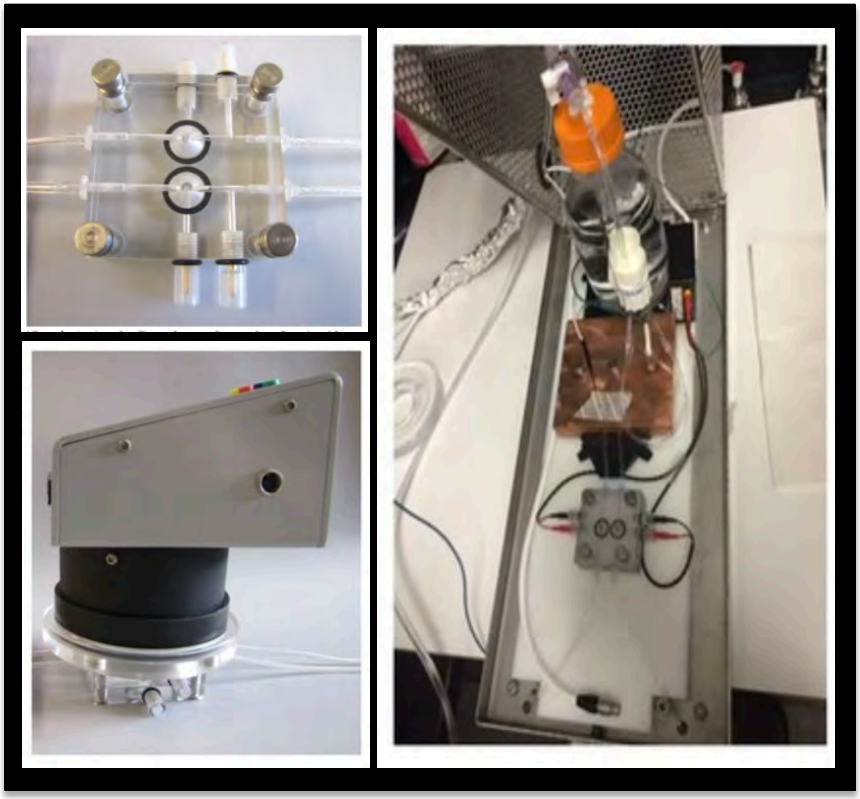


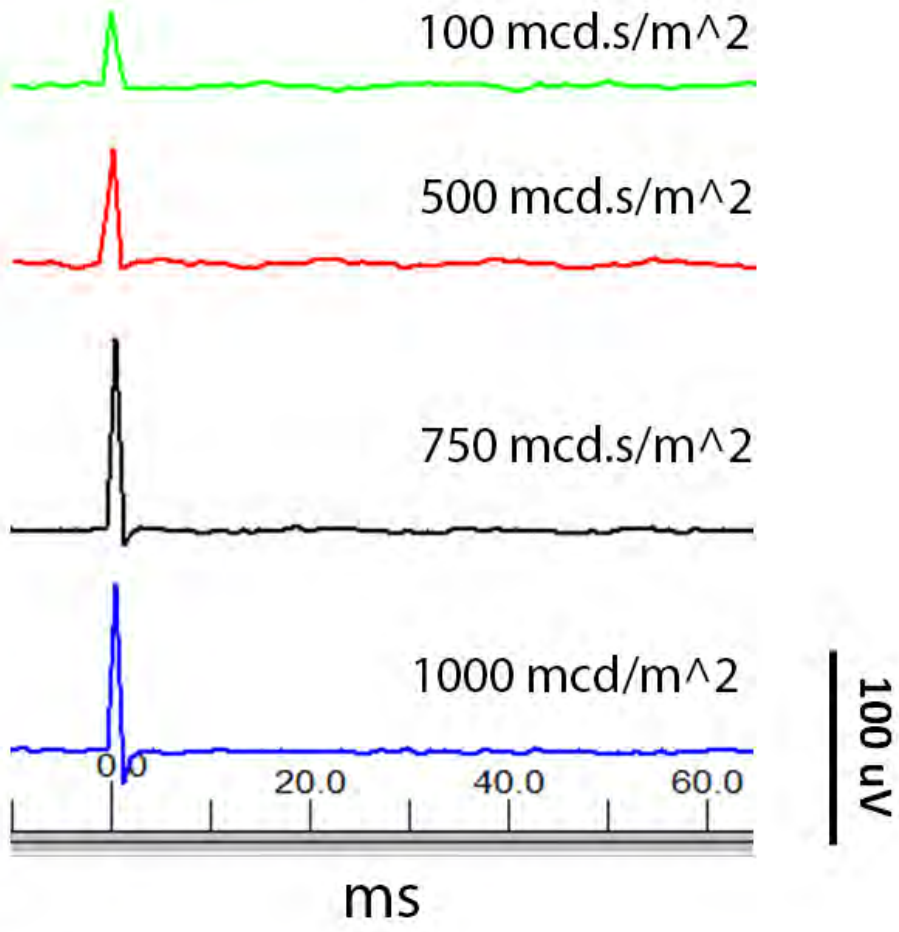






Increased retinal dysfunction within specific experimental groups





Part 1- Conclusions

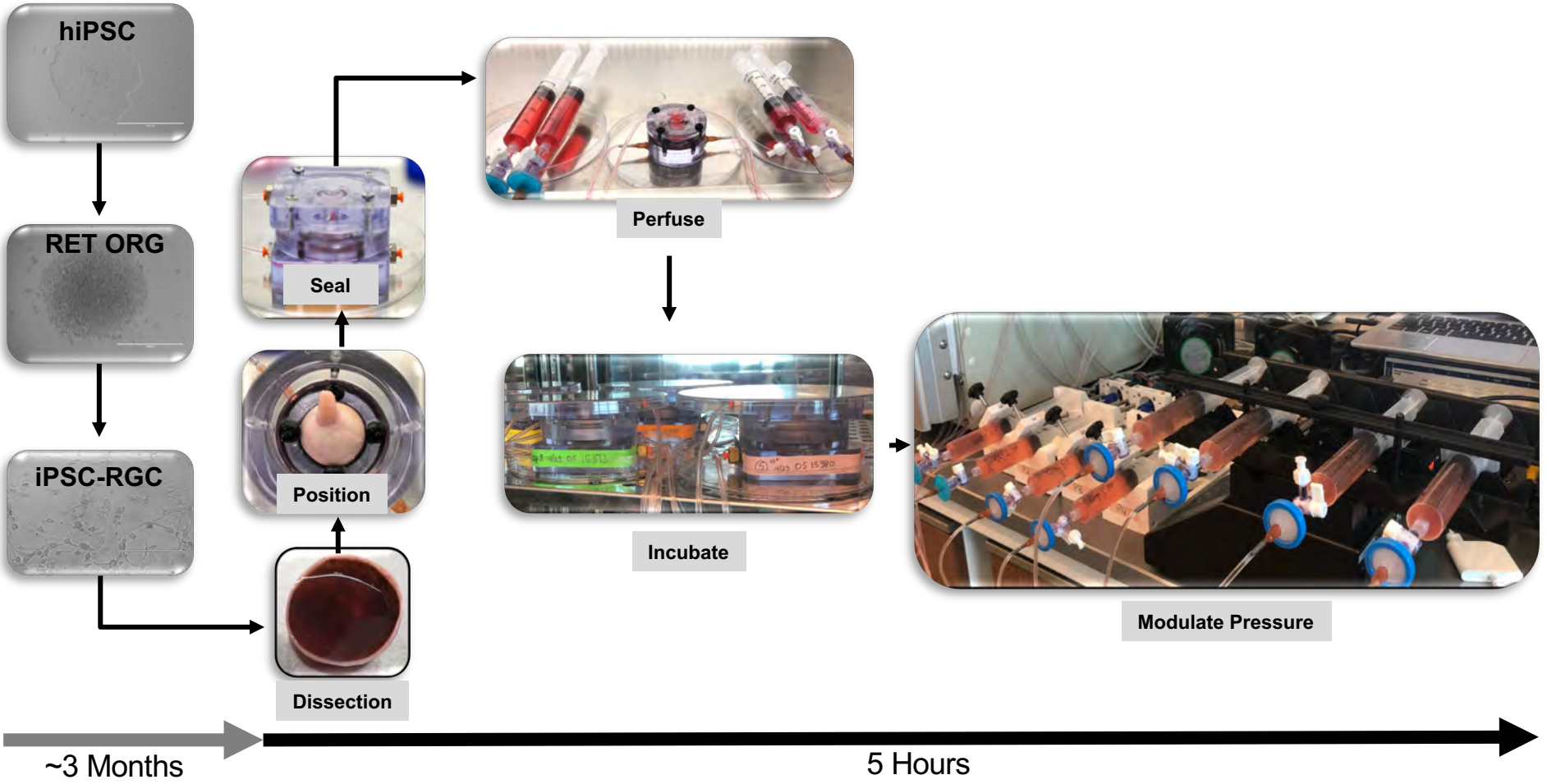
1. Effective pressure maintenance of various SANS conditions
2. Increase in width of posterior globe for all groups with insignificant height changes
3. Elevated inflammatory and apoptotic markers in ON tortuosity groups
4. Increased extracellular matrix proteins among all groups compared to control
5. Degeneration of optic nerve axons in all groups
6. Cupping, morphological reorganization and gliosis of observed in experimental groups
7. Increased TUNEL staining in mildly elevated ICP group
8. Anterograde transport of Cholera-Toxin B subunit observed in control, high translaminar pressure and ON tortuosity groups
9. Functional electroretinogram activity observed for control, groups 1 and 3

Part 1 -Future therapeutic and preclinical applications to reduce SANS risk

1. Environmental conditions:
   - Elevated CO2 concentration
   - High salt content
2. Existing therapies
3. Biomarkers
4. New Targets
5. Cell transplantation

Part 2 - Utilizing Human RGCs Seeded Posterior Cups within the TAS Model

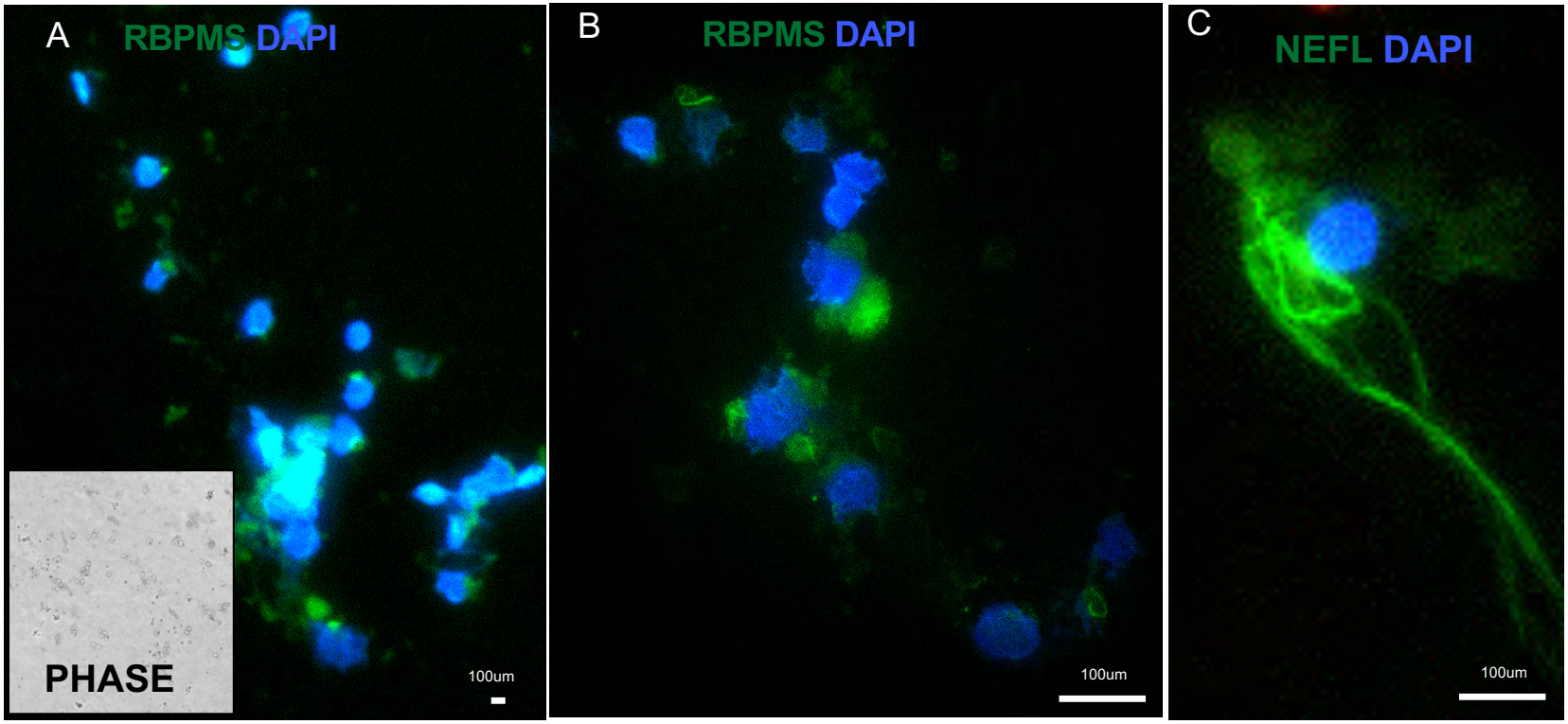
Methods



U.S. Patent Application No. 16/395,610

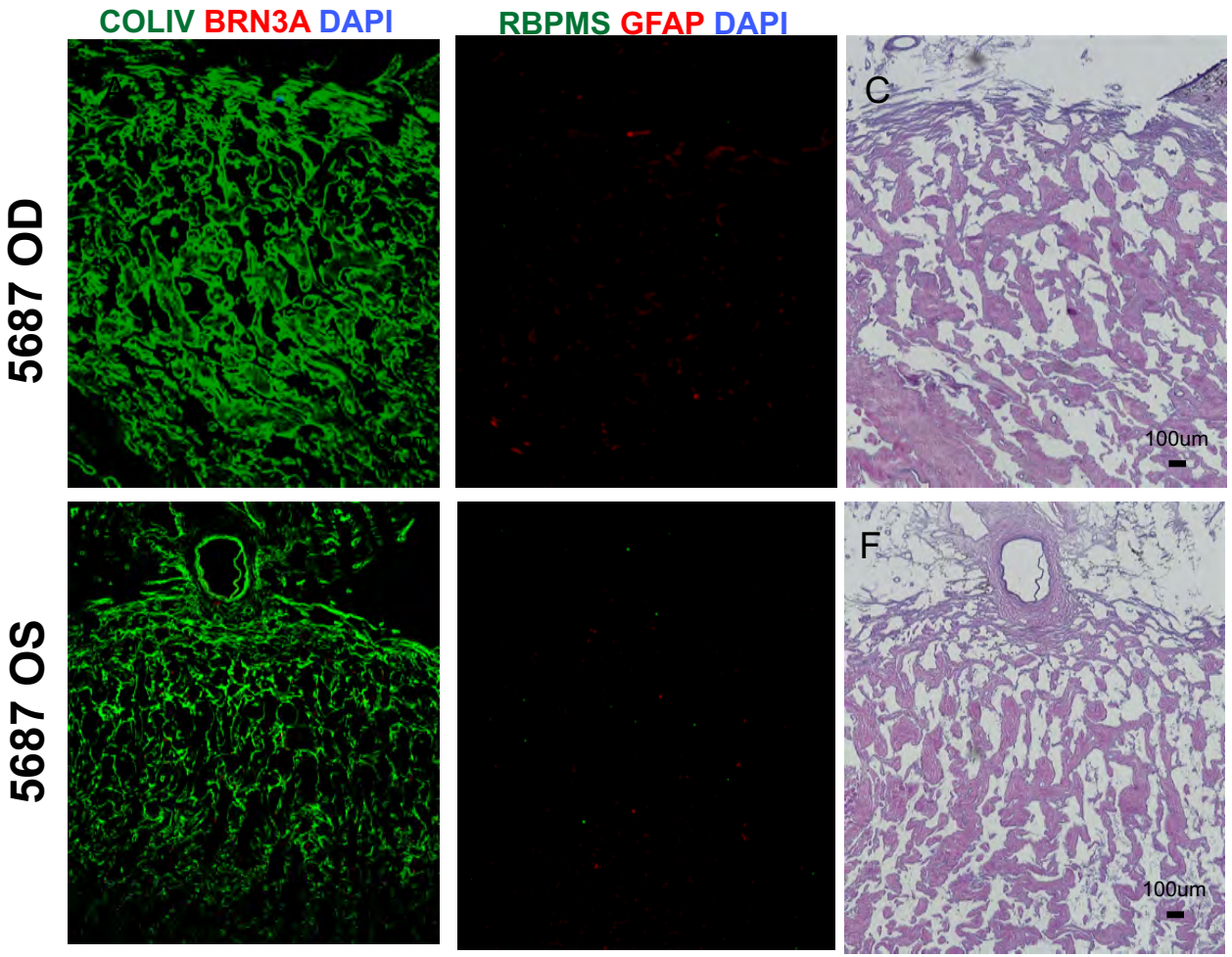
Viability of adult RGCs from native retina

Human retinal explants dissociated to culture adult human RGCs



7 days in culture

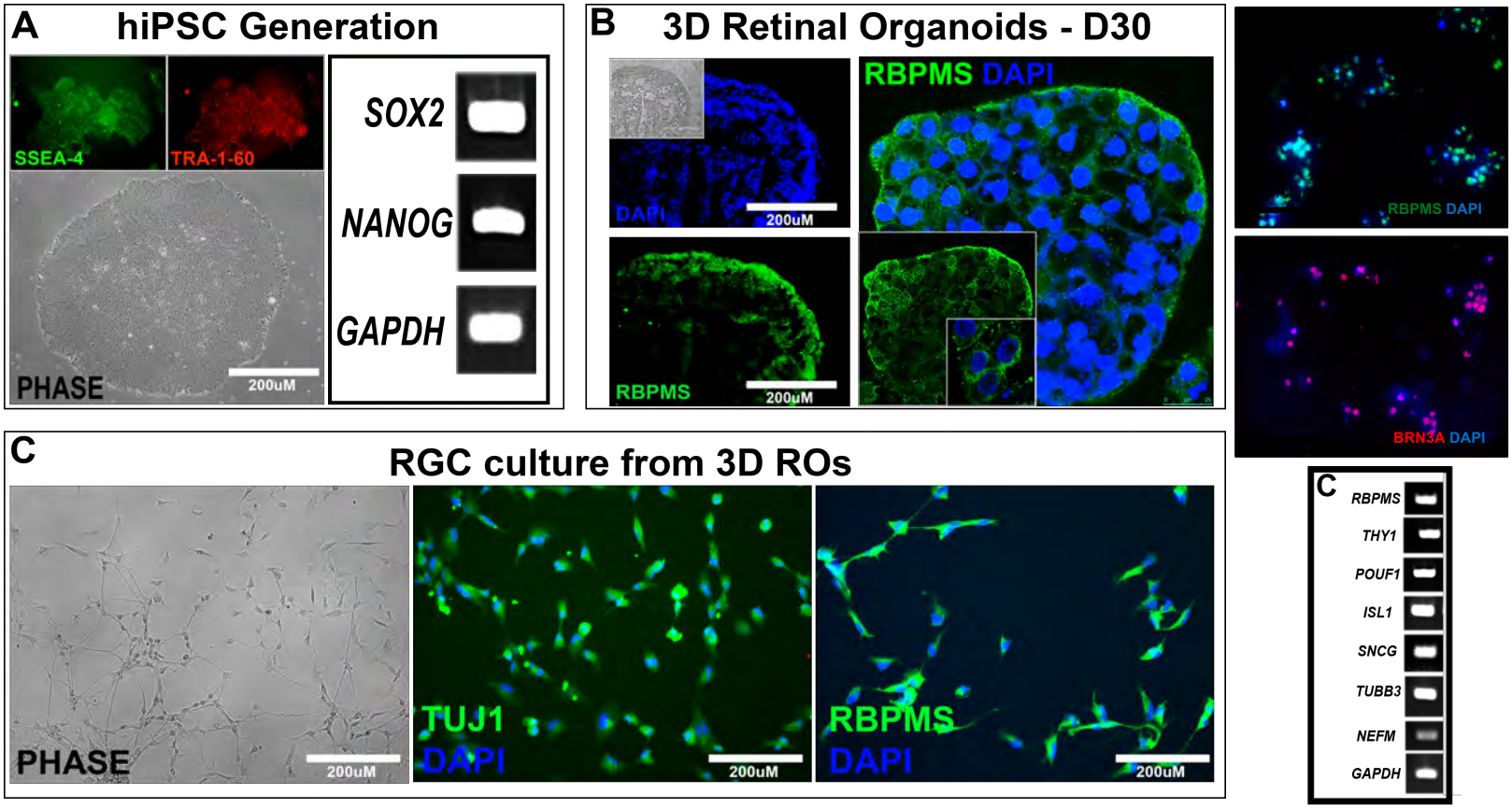
Decellularization Of Human Posterior Cups



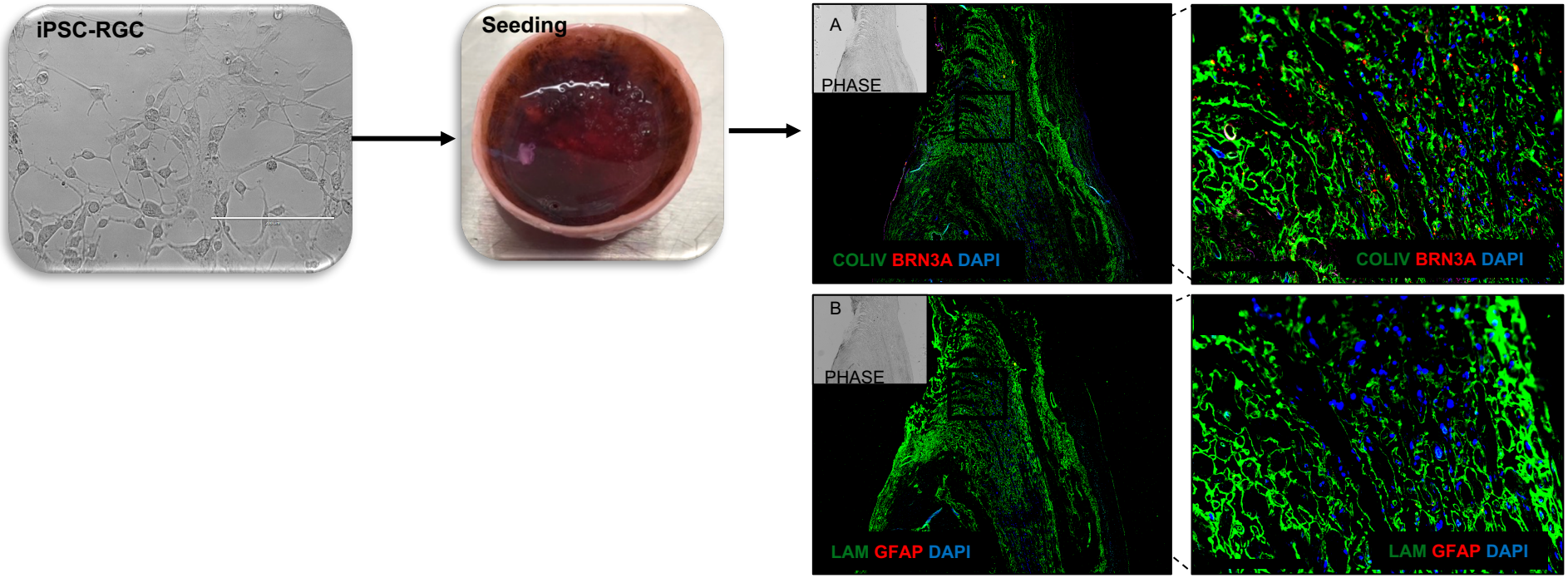
Decellularized

Decellularized
In TAS model

Generation of induced pluripotent stem cell derived retinal ganglion cells

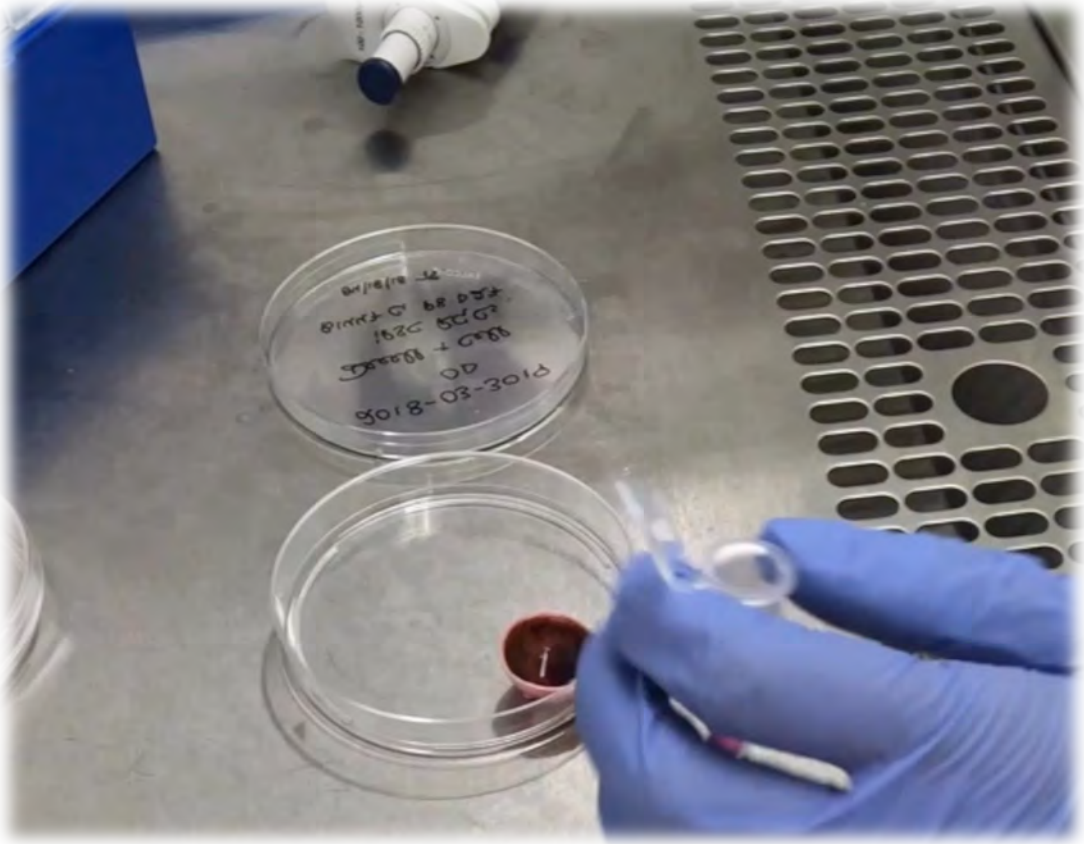


Seeding of iPSC-RGCs on Posterior Segments

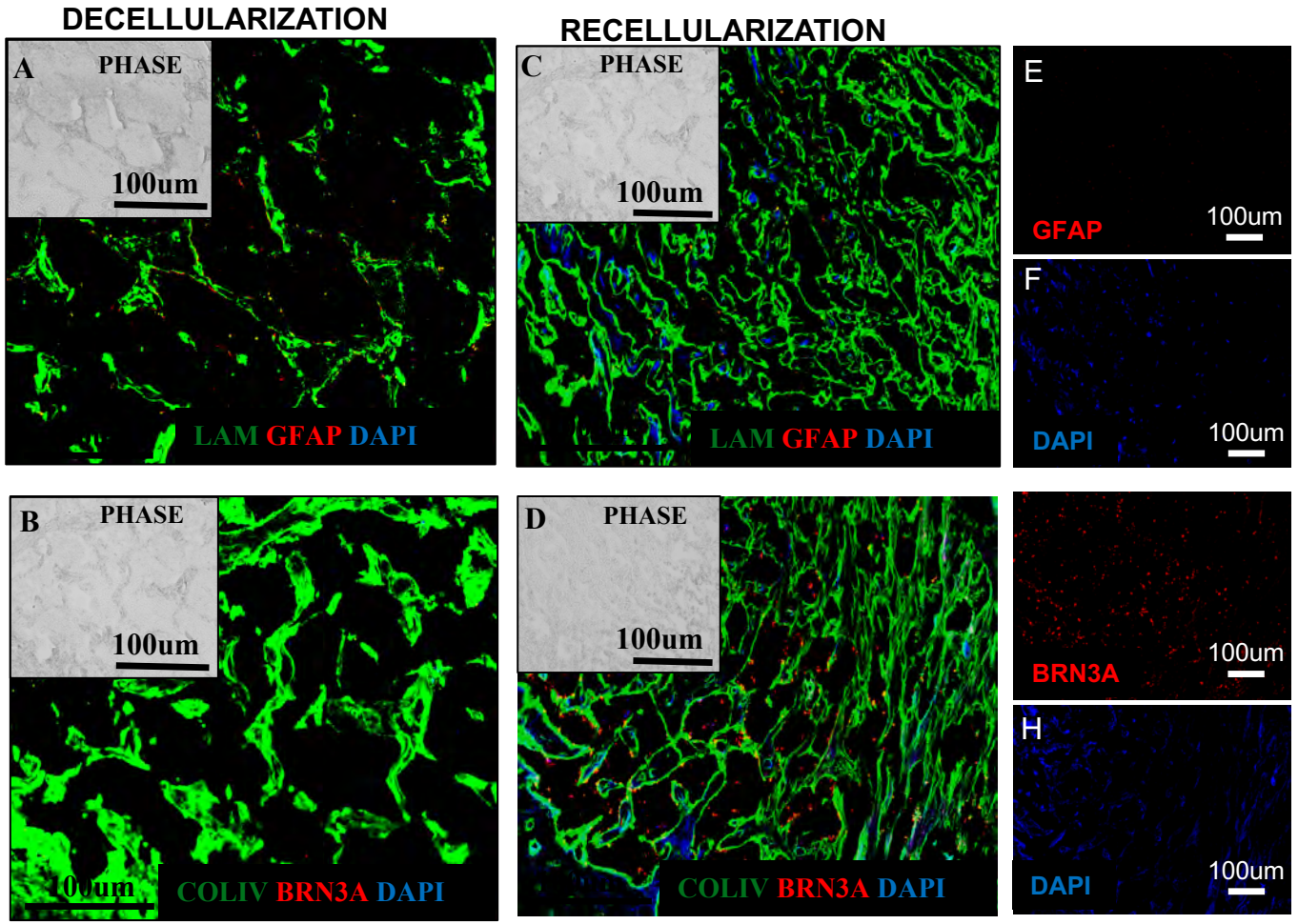


hiPSC-RGCS transplanted on the decellularized posterior cups – 7 days in culture

Seeding of iPSC-RGCs on Posterior Segments

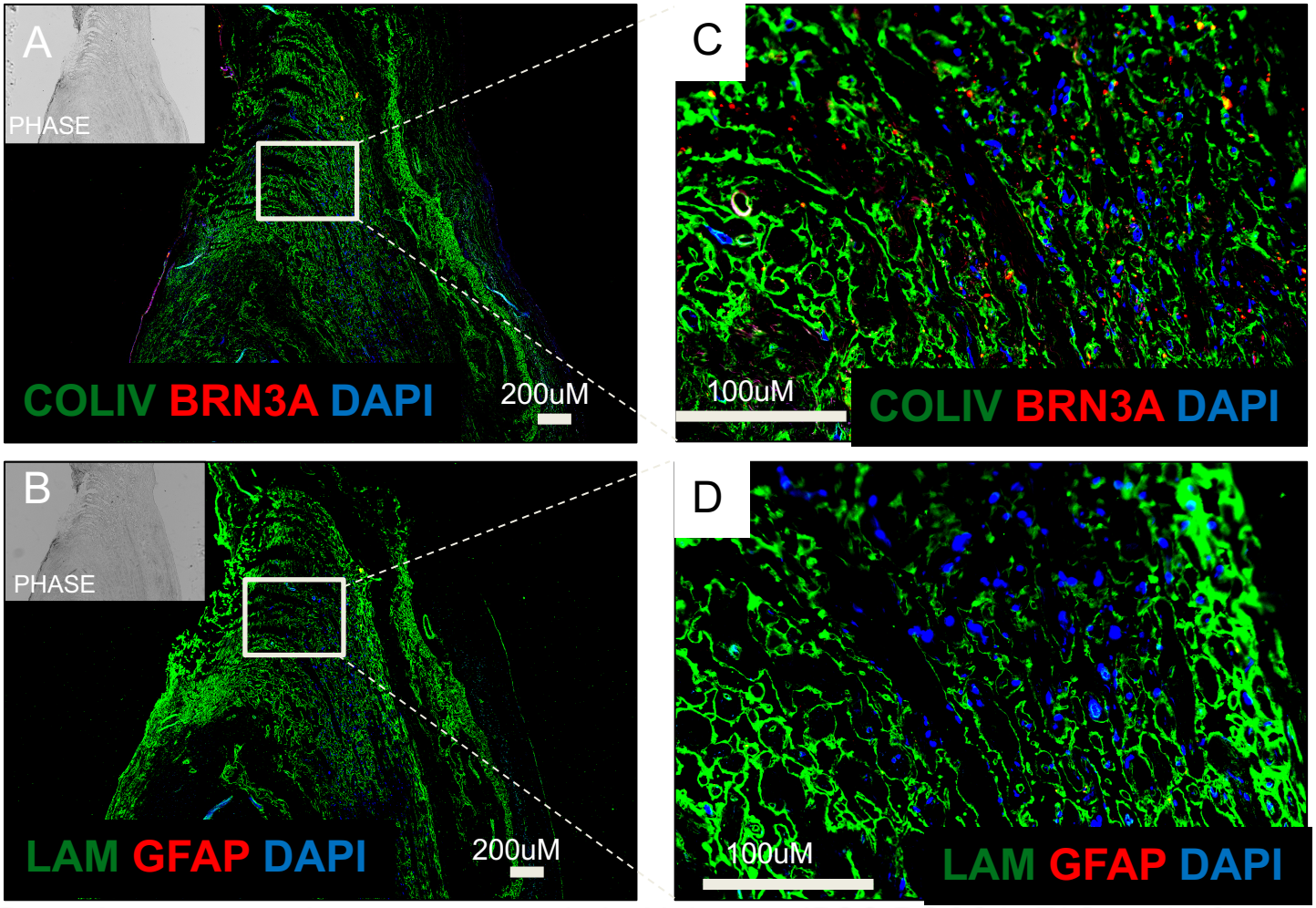


Human Posterior Cups seeded with hiPSC-RGCS in TAS Model



Donor human posterior eye cups with human iPSC-RGCS in TAS model for 7 days

Human Posterior Cups seeded with hiPSC-RGCS in TAS Model



Donor human posterior eye cups seeded with human IPSC-RGCS in TAS model for 7 days

Part 2 - Conclusions

1. Utilized combination Technologies
2. Human iPSC-RGCS
3. Reseeding of human posterior cups
4. Successful culture in Translaminar Autonomous System

Part 2 – Precision medicine-based applications of the Model

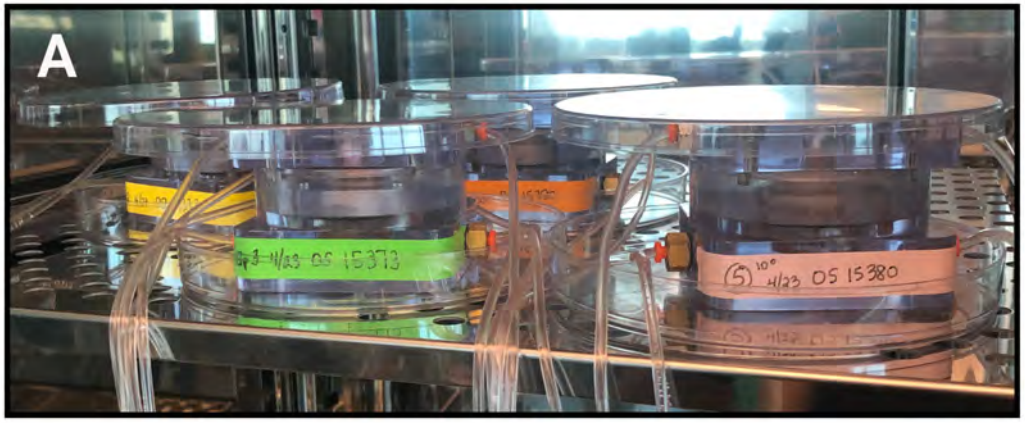
1. Seeding of human AAV2-GFP iPSC-RGCs in the donor eyes
   - Integration
   - Regenerative capacity
2. Elevated translaminar pressure gradient
   - ONH Biomechanics
   - Heterogeneity of ONH
3. Therapeutic testing in a preclinical humanized model

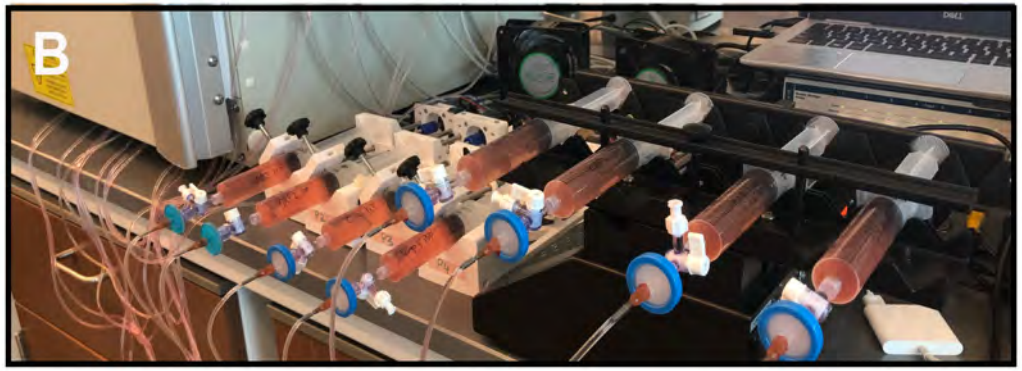
Limitations and Future Vision of the Model

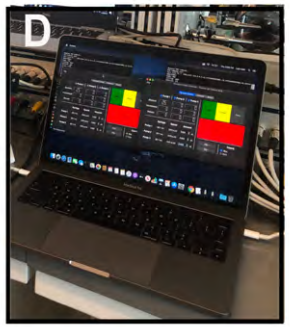
Limitations of the TAS Model

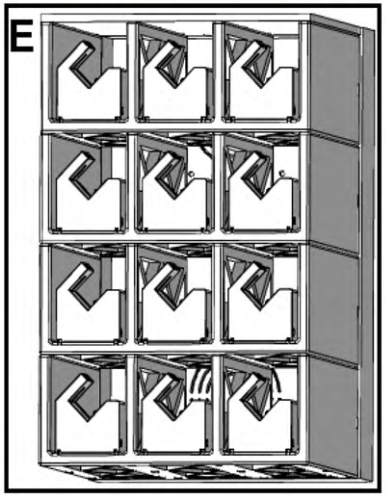
1. Variability of human donor tissue
2. Axotomized donor tissue
3. Long-term viability of donor tissue
4. Lack of vascular perfusion pressure
5. Cyclic circadian rhythms of ICP and IOP

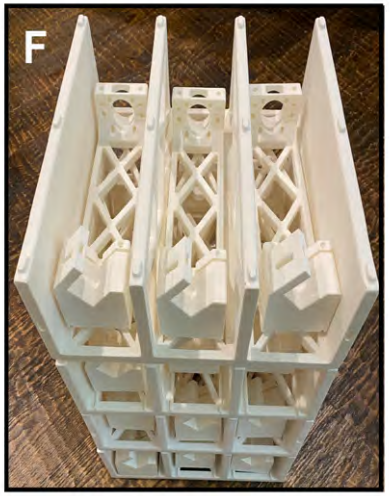
Scalability of the TAS Model







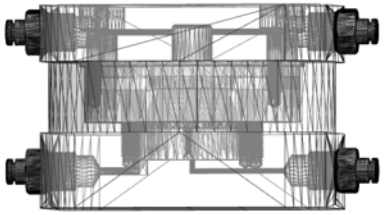


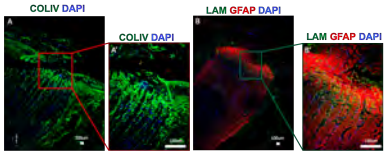


Future Directions

Therapeutic potential and preclinical applications

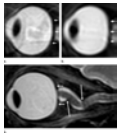
Disease Modeling	Drug Discovery	Cell Transplantation	Gene Regulation





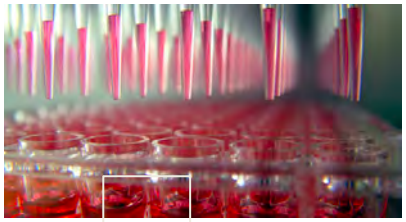
Posterior cup 3 days in TAS model

Disease Modeling



SANS

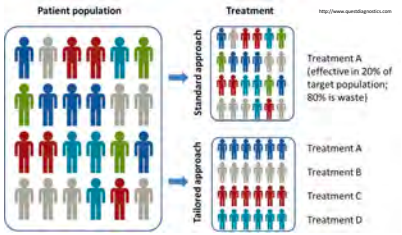
High throughput drug screening



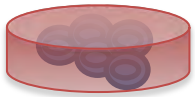
Animal disease models
Heterologous expression systems

BIOMARKERS

Precision medicine

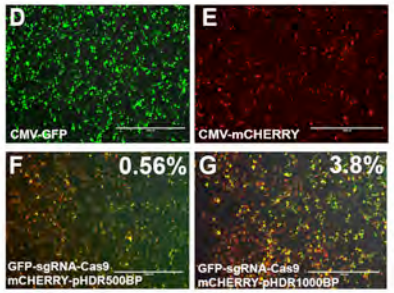


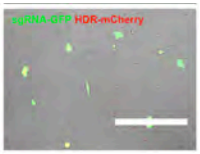
Family history
Medical history
Genetics
Lifestyle
Environment



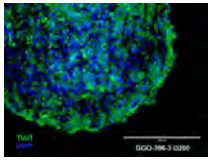
Generate control and patient-specific iPSCs

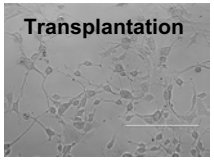
CRISPR/Cas9 based Genome editing



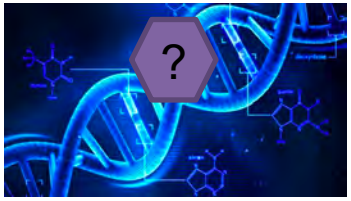


FACS

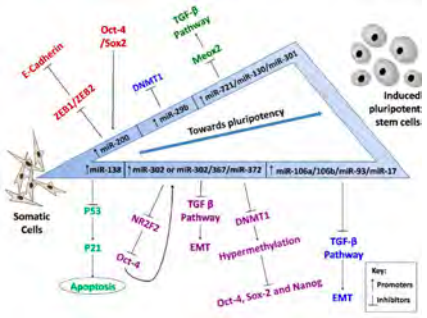




Transplantation



Understanding mechanisms of reprogramming – gene manipulation



Molecular basis of reprogramming

Translational Nature

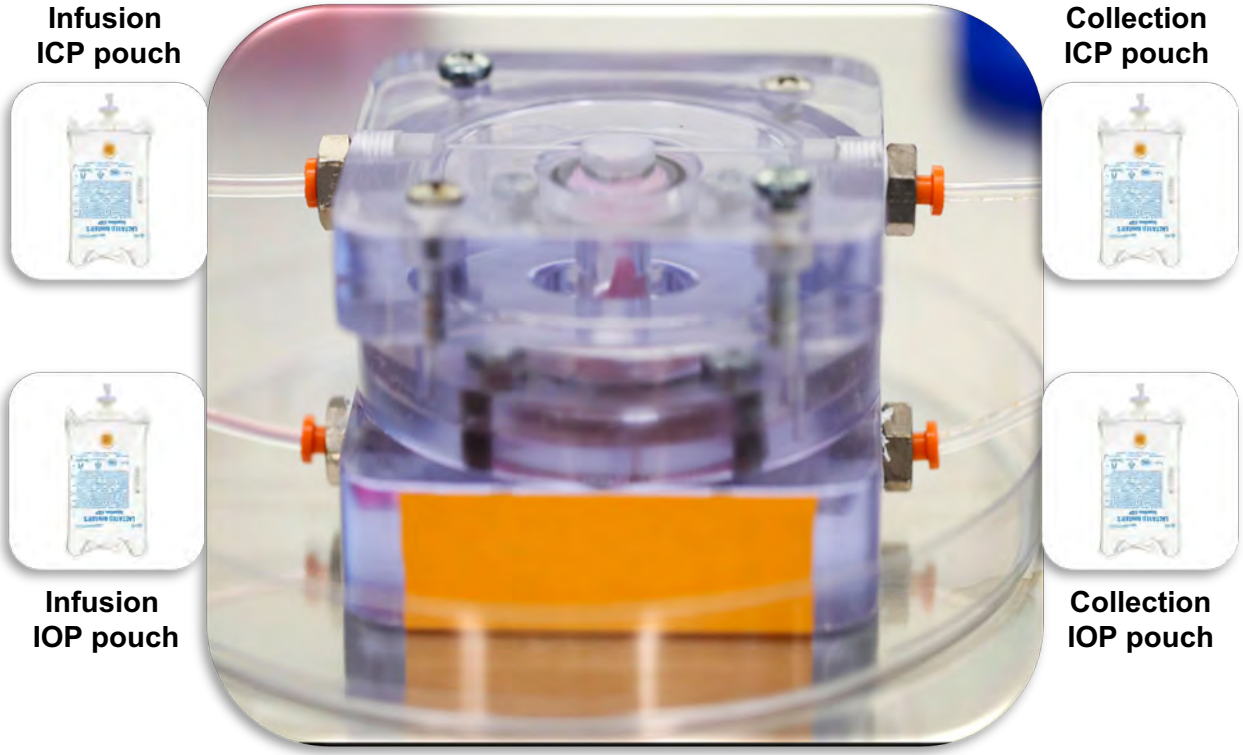
For astronauts and future space travel

1. A novel ex-vivo human preclinical SANS model
   - Ground
   - Conditions of zero and microgravity
2. Cost-effective enough to be transported
3. Test countermeasures
4. Human basis of testing – unmet clinical need for SANS
   - Identify therapies and treatments to save ganglion cells
   - Precision medicine for effective translation
   - Identify and target various biomarkers

Space Health Applications

1. Human line of testing for SANS:
   - Donor stem cells from astronauts
   - Genetic, environmental or pathogenic conditions causing SANS
   - We can compare right versus left posterior cups
2. We can effectively study other factors causing SANS:
   - Elevated levels of CO2 concentrations
   - High salt concentration medium
3. Model modified to be placed under zero-gravity chambers or taken in short-term and long-term flight studies
4. Therapies to be tested within this model
   - Medium can be collected for biomarker expression to target future therapies
   - Identify pathways or molecules that can be targeted with drugs/gene therapy
   - Animal models of ICP before translation to human clinical trials

Possible Mission to Moon and Beyond…



Acknowledgements

Indiana University School of Medicine
Weiming Mao, Ph.D.
Michael Peng
Eugene and Marilyn Glick Eye Institute
Faculty, Scholars and Staff

University Of Iowa
Budd A. Tucker, Ph.D.

University Of Wisconsin-Madison
Colleen M. McDowell, Ph.D.

Engineers
Husain Lohawala
Gaurav Sharma

University of North Texas- Health Science Center
Stacy Curry
Sandra Neubauer
Anne-Marie Brun
Yang Liu, Ph.D.
Abbot F. Clark, Ph.D.
Iok-Hou Pang, Ph.D.

Lions Eye Institute FOR TRANSPLANT & RESEARCH
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David Ammar, Ph.D.

Funding Sources:

SCHOOL OF MEDICINE
INDIANA UNIVERSITY

TRANSLATIONAL RESEARCH INSTITUTE FOR SPACE HEALTH

This work is supported by the Translational Research Institute for Space Health through NASA Cooperative Agreement NNX16AO69A