CHILDREN'S MEDICAL RESEARCH INSTITUTE

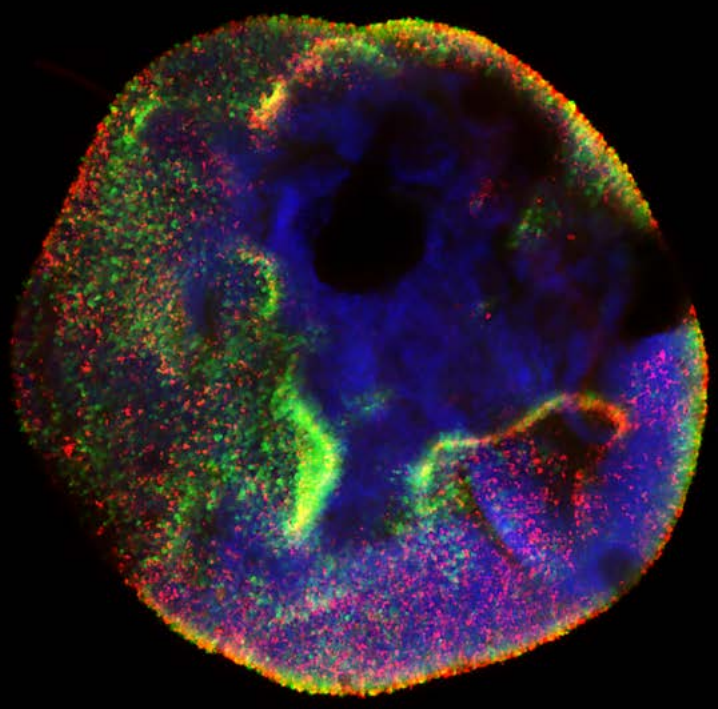
Jeans for Genes®

Stem Cell & Organoid Research at CMRI

CMRI's Stem Cell Medicine Group (SCM), led by Dr Anai Gonzalez Cordero, is focused on the development of retinal and inner ear organoids to investigate the disease pathophysiology of inherited retinal degenerations including Usher Syndrome and Stargardt's maculopathy, amongst others.

SCM has established advanced capabilities in stem cell and organoid research and are using these to understand disease mechanisms and potentially develop new cell and gene therapeutic approaches.

With the advent of induced pluripotent stem cell (iPSC) technology, it is now possible to re-create the architecture and physiology of human organs in remarkable detail. SCM aims to disseminate an increase in translational stem cell research and utilise the great potential of regenerative medicine.

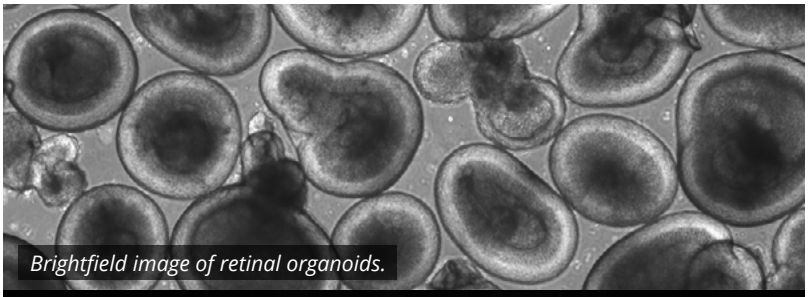


3D whole retinal organoid demonstrating rod (red) and cone (green) photoreceptors cells.

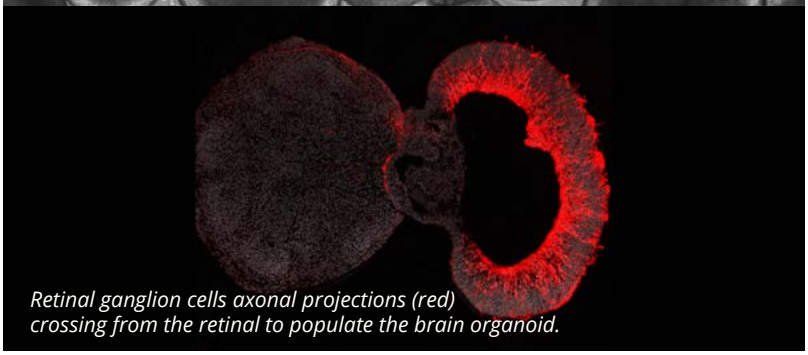
Why use organoids?

Replacement of animal models with human organoids

- Human iPSC-derived organoids better mimic human physiology and architecture.
- SCM has particular expertise in:
  - Retinal organoid generation which replicates normal retinal development. Mature retinal organoids show enhanced outer segment (OS) formation in both rod and cone photoreceptor cells, including organised stacked discs.[1]
  - Inner ear organoid generation complements research in the eye. SCM models inner ear pathophysiology in Usher syndrome organoids. The modelling of both organs enables the development of gene therapies for both blindness and deafness.
  - Retinal-brain organoids form complex organoids connected by nerve-like structure mimicking the neuronal projections that connect the eye and brain. The complex retinal-brain organoid system can facilitate the investigation of optic nerve and neurological diseases of the eye and the brain.[2]



Brightfield image of retinal organoids.



Retinal ganglion cells axonal projections (red) crossing from the retinal to populate the brain organoid.

The Future is Organoids

- FDA Modernization Act 2.0 allows alternatives to animal testing.
- EMA is implementing new measures to minimize animal testing.
- CSIRO has estimated that the revenue for generation of organoids in Australia will reach $1.28 billion by 2040.[3]

Understanding human development
SCM investigates human retinal development such as macular development using the human iPSC-derived retinal organoids.

Disease modelling
CMRI has the ability to generate robust preclinical models in which disease mechanisms can be elucidated and new therapies tested. Disease modelling organoids are generated utilising iPSCs derived from patients carrying specific mutations.

Disease modelling organoids enable:

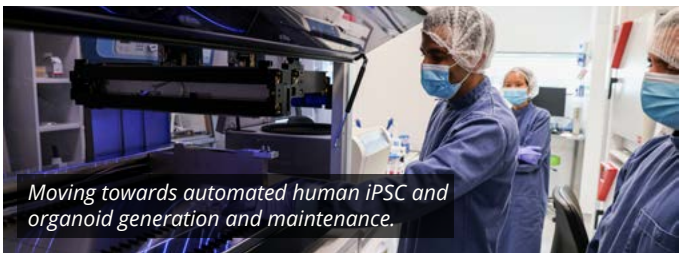
- Discovery of robust cellular phenotypes using human cells
- Generation of hundreds of human organoids for screening and omics
- Testing of new therapeutic approaches:
  - Adeno-associated virus (AAV) vector-mediated gene augmentation
  - Demonstration of phenotype rescue following treatments
  - Pharmacological screening

Advanced therapies

Drug screening
Patient-derived organoids are widely used as a screening platform for drug development. Previously, toxicity and pharmacokinetic studies relied on animal models or standard 2D models. However, these studies were limited due to species differences in anatomy, function, and morphology in various tissues.

Gene therapies
SCM has optimised the delivery of AAVs to organoids. In a proof-of-concept study X-linked RPGR organoids were utilised to demonstrate phenotype rescue following administration of AAV RPGR (ORF15) gene therapy.

Cell therapies
SCM has the ability to generate retinal organoids containing transplantable photoreceptor cells. The majority of inherited retinal diseases lead to loss of photoreceptor cells and blindness. SCM has pioneered the transplantation of photoreceptor cells for cell replacement to rescue vision. We are developing new technologies to improve the connectivity and the functionality of transplanted cells to improve transplantation outcomes.


Moving towards automated human iPSC and organoid generation and maintenance.

Affiliation

STEM CELL & ORGANOID FACILITY
THE UNIVERSITY OF SYDNEY
LUMINESCE Alliance
NSW GOVERNMENT
WE Westmead Health Precinct

Competitive advantages

High throughput automation
SCM is working towards high throughput automation of organoid generation with an in-house robot. This will significantly improve the scalability and the consistency of the organoids and research overall.

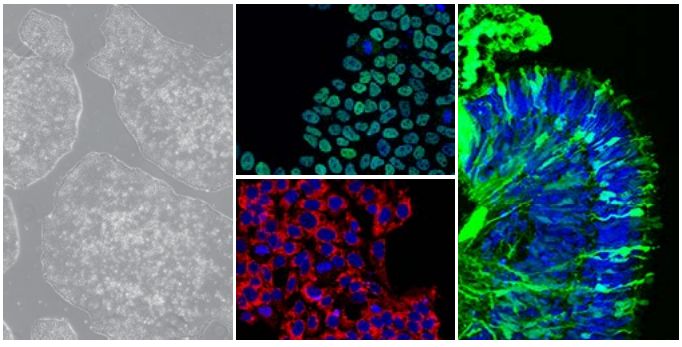
Human iPSC-derived organoids
SCM uses pluripotent stem cells (iPSC and embryonic stem cell) rather than adult multipotent stem cells. Pluripotent stem cells have the advantage of being able to differentiate into any interested cell type while adult stem cells have limited differentiation ability.

Dedicated human organoid facility
A dedicated Stem Cell and Organoid Facility (SCOF), which offers iPSC reprogramming service and a range of human iPSC-derived 2D and 3D cell types for research. SCOF offers a range of central nervous system organoids such as cortical organoids, whole cerebral organoids, and cortical-retinal organoids.

Functional assays
High throughput state of the art micro electrode array (MEA) to assess excitable cell function.

Know-how

- Established capabilities in the utilisation of retinal organoids for gene therapy studies.
- Optimised protocols for retinal organoids proteome and single-cell RNA sequencing.


Images (left to right): *Brightfield images of human iPSCs, human iPSCs showing pluripotency markers NANOG (top) and LIN28 (bottom) and retinal organoids transduced with a novel AAV variant.*

[1] Antioxidant and lipid supplementation improve the development of photoreceptor outer segments in pluripotent stem cell-derived retinal organoids. West EL, Majunder P, Naeem A, Fernando M, O'Hara-Wright M, Lanning E, Kloc M, Ribeiro J, Ovando-Roche P, Shum IO, Jumbu N, Sampson R, Hayes M, Bainbridge JWB, Georgiadis A, Smith AJ, Gonzalez-Cordero A, Ali RR. 2022, Stem Cell Reports. 2022 Mar 15:S2213-6711(22)00132-1. Doi: 10.1016/j.stemcr.2022.02.019

[2] Differentiation of brain and retinal organoids from confluent cultures of pluripotent stem cells connected by nerve-like axonal projections of optic origin. Milan Fernando, Scott Lee, Jesse R. Wark, Di Xiao, Hani J. Kim, Grady C. Smith, Ted Wong, Erdahl T. Teber, Robin R. Ali, Pengyi Yang, Mark E. Graham, Anai Gonzalez-Cordero 2022, Stem Cell Reports; doi.org/10.1016/j.stemcr.2022.04.003

[3] https://www.csiro.au/en/nonanimalmodels

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