HAL
open science

Promoting the functional maturation of human iPS cell-derived retinal ganglion cells with a 3D/2D stepwise differentiation protocol

Oriane Rabesandratana, Antoine Chaffiol, Amélie Slembrouck-Brec, Sacha Reichman, Céline Nanteau, Giuliana Gagliardi, Jens Duebel, Olivier Goureau, Gael Orieux

To cite this version:

Oriane Rabesandratana, Antoine Chaffiol, Amélie Slembrouck-Brec, Sacha Reichman, Céline Nanteau, et al.. Promoting the functional maturation of human iPS cell-derived retinal ganglion cells with a 3D/2D stepwise differentiation protocol. Association for Research in Vision and Ophtalmology_ARVO_annual congress 2017, May 2017, Baltimore, United States. hal-03495768

HAL Id: hal-03495768

https://hal.science/hal-03495768v1

Submitted on 20 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Promoting the functional maturation of human iPS cell-derived retinal ganglion cells with a 3D/2D stepwise differentiation protocol

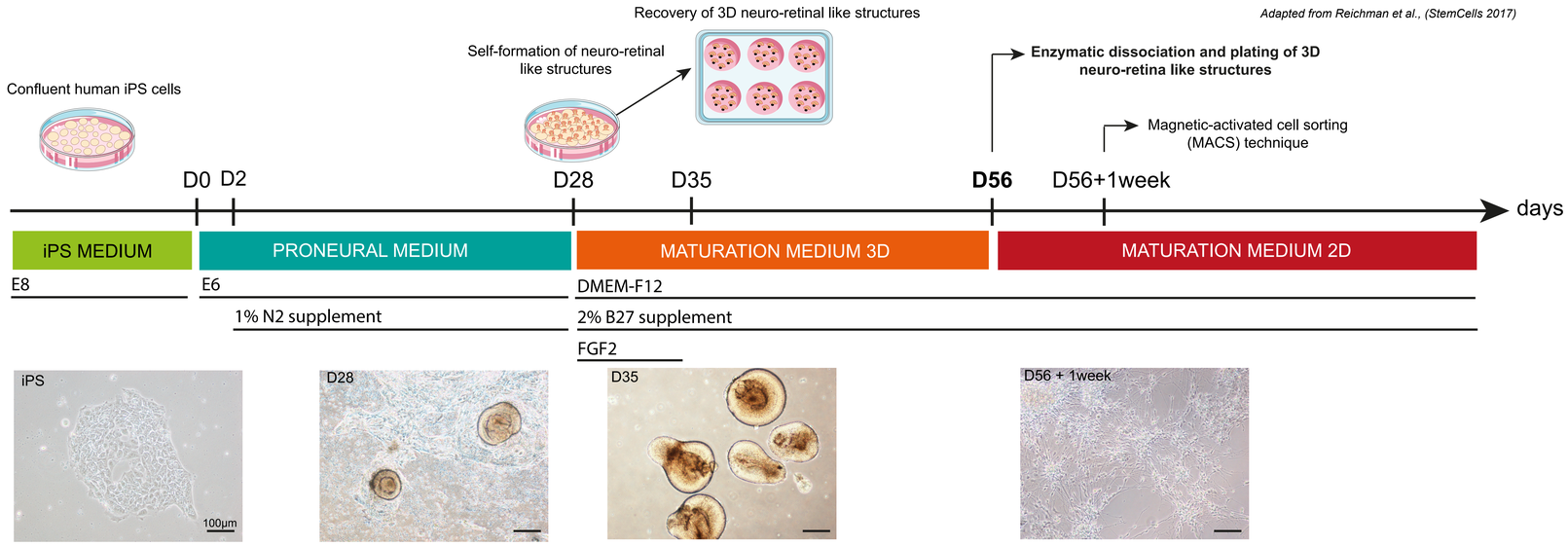
O. Rabesandratana, A. Chaffiol, A. Slembrouck, S. Reichman, C. Nanteau, G. Gagliardi, J. Duebel, O. Goureau, G. Orieux

Institut de la Vision, Sorbonnes Universités, UPMC - Univ Paris 06, INSERM U968, CNRS UMR 7210, Paris, France

Optic neuropathies have currently no effective treatment to stop the progressive loss of retinal ganglion cells (RGCs) and the degeneration of the optic nerve. Stem cell-based therapy using human induced pluripotent stem cells (hiPSCs) is one of the most promising strategies to replace lost RGCs.

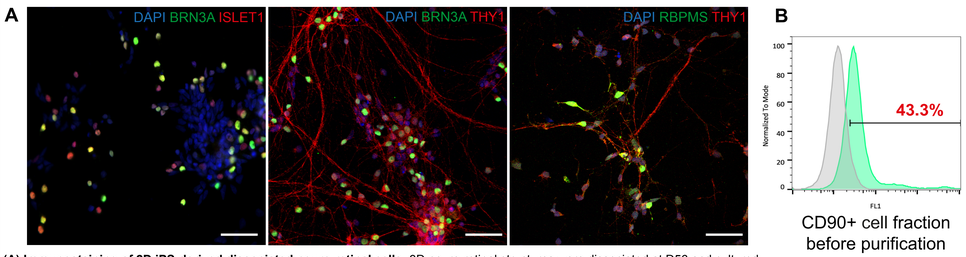
The present work displays the optimized generation and characterization of hiPSC-derived RGCs, based on our previous retinal differentiation protocol using adherent hiPSCs (Reichman et al., *StemCells* 2017).

Here, a new differentiation protocol was developped, based on two major steps in order to promote the generation of functional hiPSC-derived RGC : firstly the isolation and the culture of 3D neuro-retinal structures and secondly the culture on adherent coating of dissociated neuro-retinal cells prior to magnetic cell sorting based on CD90/Thy1 expression .



In vitro characterization of human iPSC-RGCs

Unsorted hiPSC-derived RGCs

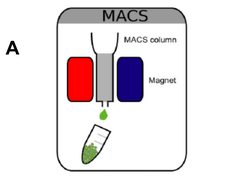


CD90+ cell fraction before purification

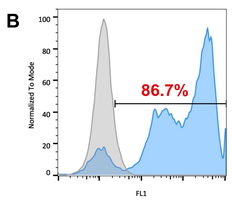
(A) Immunostaining of 2D iPS-derived dissociated neuro-retinal cells: 3D neuro-retinal structures were dissociated at D56 and cultured onto poly-D-lysin/laminin coated plates for 1-2 weeks before immunostaining. Scale bars = 50µm.
(B) Before MACS, the cell population consists of 43,3 % CD90(Thy1)-positive cells.

→ **The culture of dissociated neuro-retinal organoids on coated plates promotes neurite growth from cells expressing RGC specific markers.**

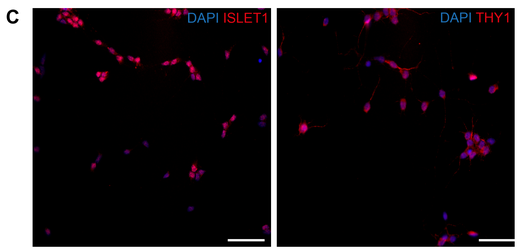
MAC-sorted hiPSC-derived RGCs



Adapted from Santos-Ferreira et al. (Front. Syst. Neurosci. 2017)



CD90+ cell fraction after purification



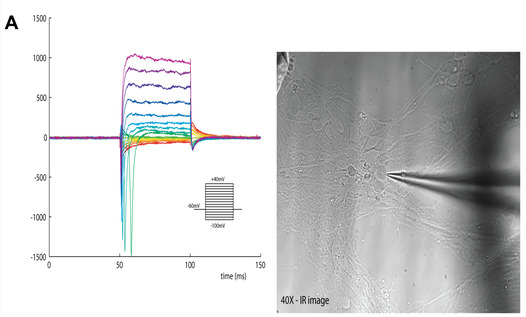
(A) Purification of hiPSC-derived RGCs: Magnetic-activated cell sorting (MACS) was performed by using anti-CD90 microbeads.
(B) Cytofluorometry analysis: Sorted CD90+ fraction showed a 86.7% CD90+ cell population.
(C) Immunostaining of sorted CD90+ 2D iPS-derived dissociated neuro-retinal cells: CD90+ 2D neuro-retinal cells were cultured onto coated plates for 1 week before immunostaining. Scale bars = 50µm.

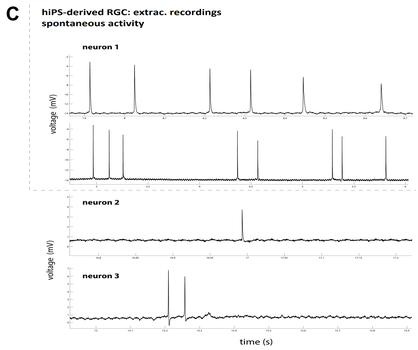
→ **Cytofluorometric analysis revealed an 86.7% enrichment of RGCs based on CD90 expression after MACS. Sorted CD90+ cells also exhibited other RGC markers expression, which would demonstrate an RGC profile.**

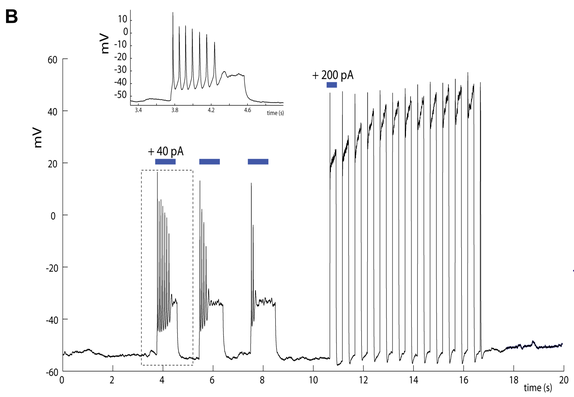
Aknowledgements Cytofluorometry platform, Institut de la Vision

Functional characterization of human iPSC-RGCs

Electrophysiological recordings of 5 weeks post-dissociation







Patch-clamp recordings
Dissociated hiPSC-derived neuro-retinal cells from 3D structures were cultured 5 weeks post dissociation, before electrophysiological recordings.

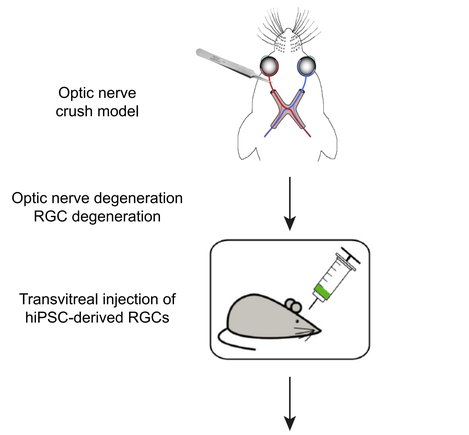
(A) current response to increasing voltage step (-100 to +40 mV) (n=7).
(B) Action potentials were evoked by a depolarizing current injection (+40pA from the resting membrane potential) (n=3).
(C) Spontaneous activity was detected in some neuro-retinal cells (n=3).

→ **In vitro, some hiPSC-derived neuro-retinal cells exhibited a functional RGC profile by electrophysiological recordings (voltage-gated current in A; spontaneous or evoked spikes in B and C), at a stage of 5 weeks after cell dissociation and plating in adherent coating.**

Discussion

Our results support the conclusion that the differentiation and the isolation of hiPSCs-derived RGC is facilitated by the sequential two step culture (3D/2D). The culture of hiPSC-derived neuro-retinal cells on coated plates stimulate the growth of Thy1-positive neurites from RGC allowing the purification of this cell population, a required step for future transplantation studies and contribute to the functional maturation of RGC as demonstrated by the electrophysiological investigations.

In vivo characterization of human iPSC-RGCs



Acknowledgements: This work was supported by grants from the ANR [GPiPS: ANR-2010-RFCS005], RETINA France and UNADEV-Aviesan (Maladies de la vision: origines et traitements). It was also performed in the frame of the LABEX LIFESENSES [ANR-10-LABX-65] supported by the ANR within the Investissements d'Avenir program [ANR-11-IDEX-0004-02]. O. Rabesandratana is supported by the Federation des Aveugles et amblyopes de France. We thank L. Ranchio (Cytofluorometry platform, Institut de la Vision) for FACS analysis.