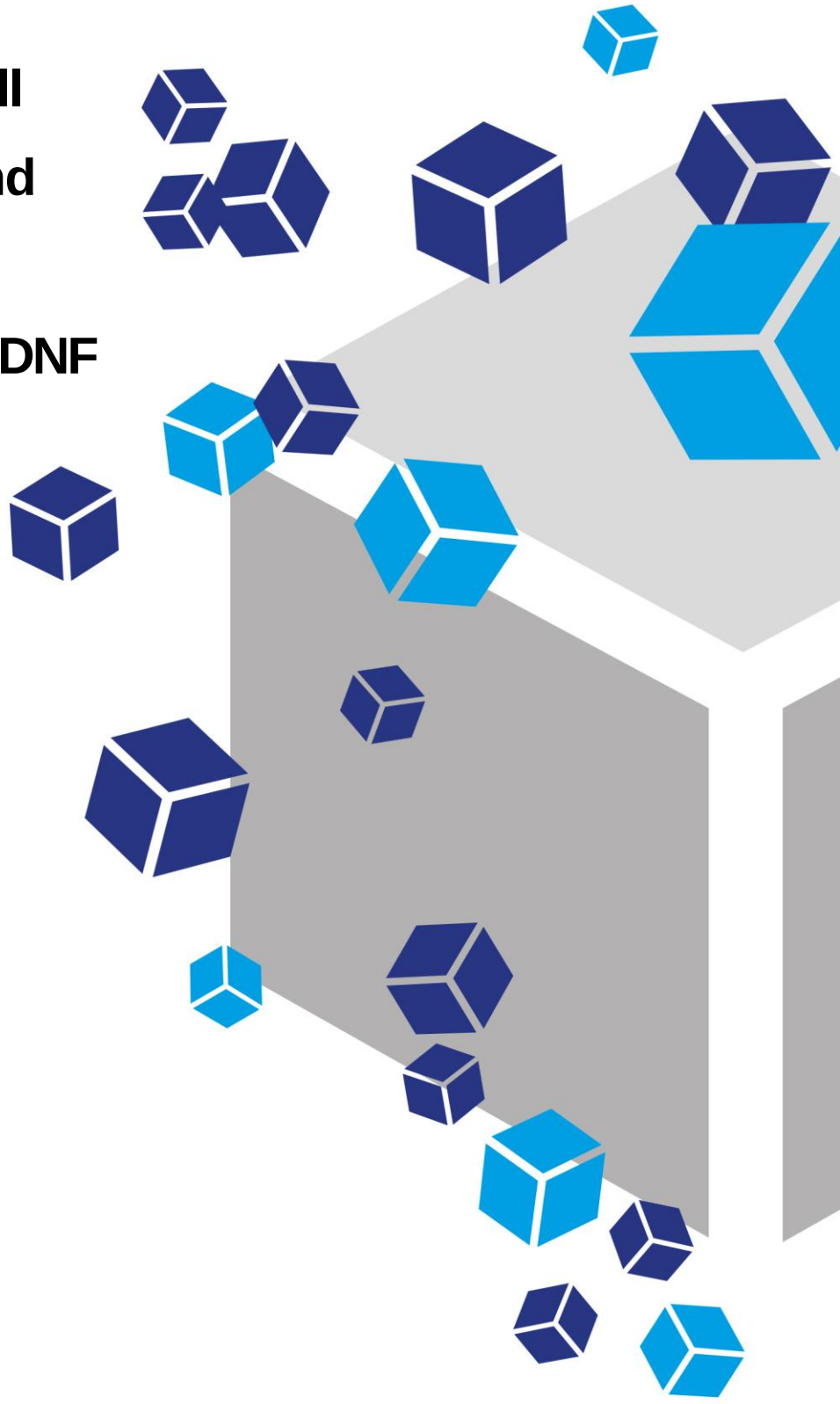
# Application Note

## Retinal ganglion cell organoid culture and transplant utilizing PODS® BDNF and GDNF



# Retinal ganglion cell organoid culture and transplant utilizing PODS® BDNF and GDNF

**Data Courtesy of Julia Oswald and Petr Baranov**
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## The challenge for conventional growth factors

Many proteins, especially growth factors and cytokines, when used as a reagent, degrade quickly, rapidly losing their bioactivity. Additionally, they can also suffer from lot-to-lot product variation. This fragility and variability hampers research and significantly limits the therapeutic potential of proteins.

## Protein Micro-depots

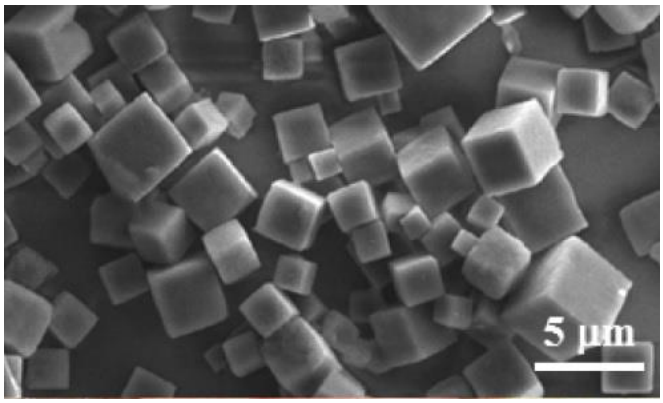
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

## Introducing PODS®

PODS® is a sustained release system which continuously replenishes proteins from millions of local microscopic stores which can be placed next to (or at a distance from) cells, either randomly or in precise locations. Just like cells, these micro-depots release a steady stream of bioactive protein. This protein can be limited to local surroundings or dispersed more widely, or made to form a gradient.

## How does it work?

At the heart of PODS® is an extraordinary polyhedrin protein. This specific polyhedrin protein has the unique ability to encase cargo proteins within perfect, transparent, cubic, micro-sized crystals, much smaller than the cells. These protein crystals form admixtures of the polyhedrin and cargo proteins which slowly degrade, releasing the biologically active cargo protein.




## How can PODS® help my research?

PODS® are tough and will withstand physical and chemical stress, so you can handle them with ease. PODS® typically release intact cargo protein over several weeks and months. Using PODS® you can readily create a steady-state protein environment in microscopic detail wherever you want, tailored exactly to your requirements. This is the power of PODS®. PODS® proteins are now available for many growth factors and cytokines and are already being used in many leading world-class research labs. PODS® protein applications include:

- Micropatterning
- Physiological, stable gradient formation
- Bioinks for 3D printing
- Microcarriers
- Functionalizing scaffolds
- Microfluidics (lab on a chip)
- Improved and simplified stem cell culture
- Therapeutic protein delivery

# Overview

Retinal ganglion cells (RGCs) conduct visual signals from the eye to the brain. Damage and degradation of RGCs occurs in many diseases leading to vision loss. Cell therapy to replace lost cells has potential to repair this damage. For this approach to succeed, the transplanted cells must be of sufficient quality to survive transplantation and mature into functional cells with axons of sufficient length to allow re-connection of the eye to the brain.

Implanted cells must be produced consistently at high quality and be able to adapt from the *in vitro* conditions of cell culture to the *in vivo* environment within the eye. PODS® sustained release has potential to simplify and improve the process of generating cells and, since they provide long-term sustained release of growth factors, also to act as a survival agent enabling engraftment and promoting maturation of implanted cells.

The data presented here illustrate the significant improvements that can be achieved in neuronal culture, both *in-vitro* and *in-vivo*, using PODS® BDNF and PODS® GDNF nanocrystals to produce RGCs and engraft RGCs.

# Methods

For RGC organoid formation (Fig 1), mouse embryonic stem cells (mESCs) were first cultured in optic vesicle medium in a V-bottom 96 well plate and transitioned a petri dish containing optic cup medium from day 9 (D9). PODS® growth factors were introduced to the culture system on day 20 by single addition of $8x10^6$ PODS® BDNF and $8x10^6$ PODS® GDNF crystals. NOTE: During the 10-day period of PODS® growth factor treatment, no new PODS® were added and only a single half media change was performed.

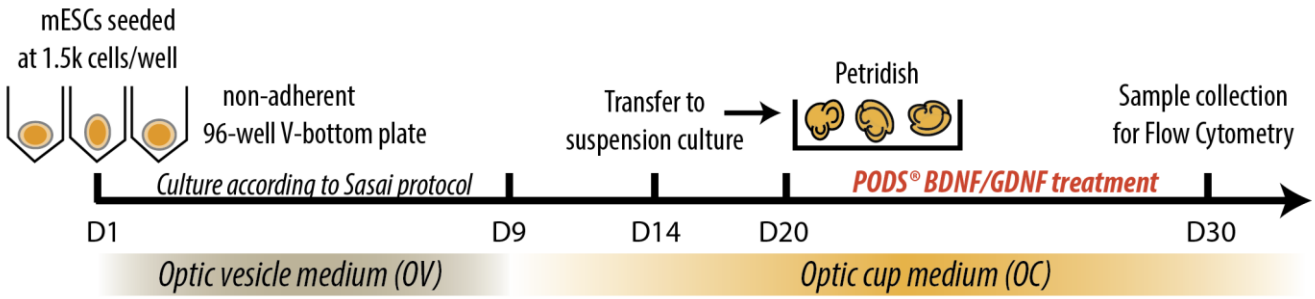



**Figure 1. Schematic of the culture system used to prepare retinal organoids.**

RGC organoids were processed, analyzed and implanted as shown in Figure 2. At D21, following formation of optic cups, organoids were dissociated into single cells. The single cells were passed through a 40 µm filter mesh and RGCs immune affinity selected using magnetic microbeads coated with Thy1 antibodies. To assess their quality, the selected cells were analyzed by staining with a series of three labelled antibodies against RGC markers. (1) RNA-binding protein with multiple splicing (RBPMS), a general marker (2) melanopsin to identify photosensitive RGCs and (3) osteopontin to identify alpha-RGCs. Cells were subjected to flow cytometry on a MACSQuant instrument.

For implantation into each mouse eye, 20,000 RGCs were resuspended in 2 µl PBS and injected intravitreally either without PODS® or after combining with $10^5$ PODS® BDNF and $10^5$ PODS® GDNF nanocrystals. Following injection, the mice were maintained for a period of two weeks after which they were sacrificed and the eyes subjected to histological analysis to assess RGC engraftment. Thy1 was used to detect engrafted RGCs and β3-tubulin (TUJ-1) used as general neuronal marker

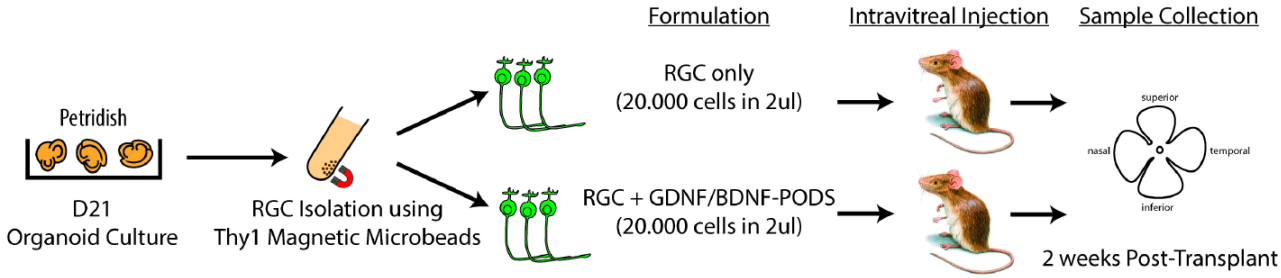



**Figure 2. Schematic of the processing of optic cups into single cells and intravitreal injection.**

# Results

RGC organoids were generated by a single application of PODS® crystals on D20 and a half media change on D25. Using conventional BDNF/GDNF, at least 250 ng of each GF had to be added five times during the 10-day culture period to achieve a similar effect (data not shown). Consistent with higher quality, organoids had smoother surfaces when incubated with PODS®.

At D30, RGCs were dissociated into single cells, collected using immuno-affinity capture, stained with RBPMS (pan-RGC marker), melanopsin (photosensitive RGC marker) and osteopontin (alpha-RGC marker) and subjected to FACS analysis. As shown in Figure 3, RGCs grown in the presence of PODS® crystals were significantly enriched for RGCs expressing each of the three markers.

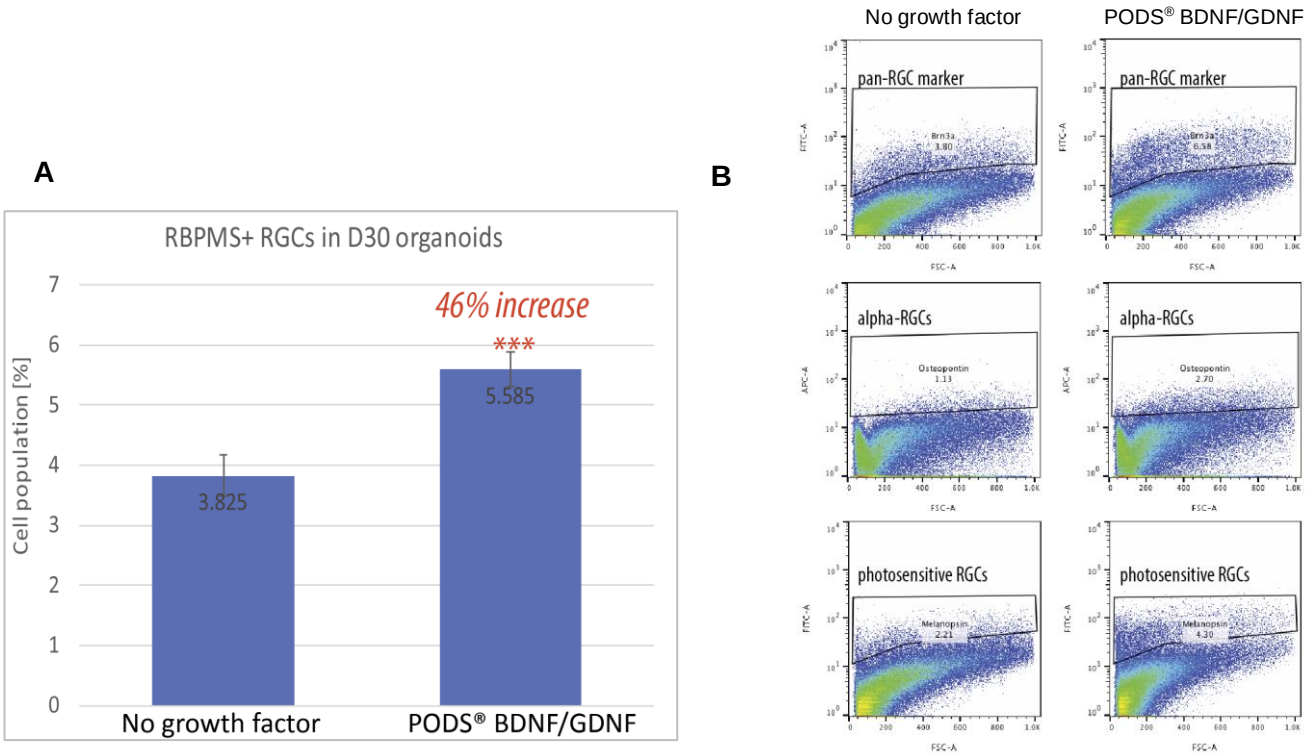



**Figure 3. Quantification of RGC sub-types.** 3D-retinal organoids were cultured for 10 days either in the absence (left column) or presence of PODS® BDNF and PODS® GDNF crystals (right column). **(A)** Quantification of total RGCs with the neurochemical marker RBPMS. RGC data plotted as percentage of total cell number (n=4). **(B)** FACS analysis of cells stained with RPMI (pan-RGC), osteopontin (alpha-RGCs) and melanopsin (photosensitive RGCs). Analysis of FACS data shows yields of each cell subtype was increased up to 2-fold by the addition of PODS® growth factors.

Subsequently, dissociated RGCs were transplanted by intravitreal injection into mouse eyes either in the presence or absence of PODS® BDNF and PODS® GDNF. Two weeks following implantation, eyes were dissected and stained with Thy1 and β3-tubulin to reveal implanted cells (green) against the background of existing neuronal cells (red). The images shown in Figure 4 are representative of results seen across 5 eyes tested for each condition (with or without PODS®). Not only was the number of cells which survived significantly higher with PODS®, the levels of maturation was remarkably higher with axon extensions more pronounced.

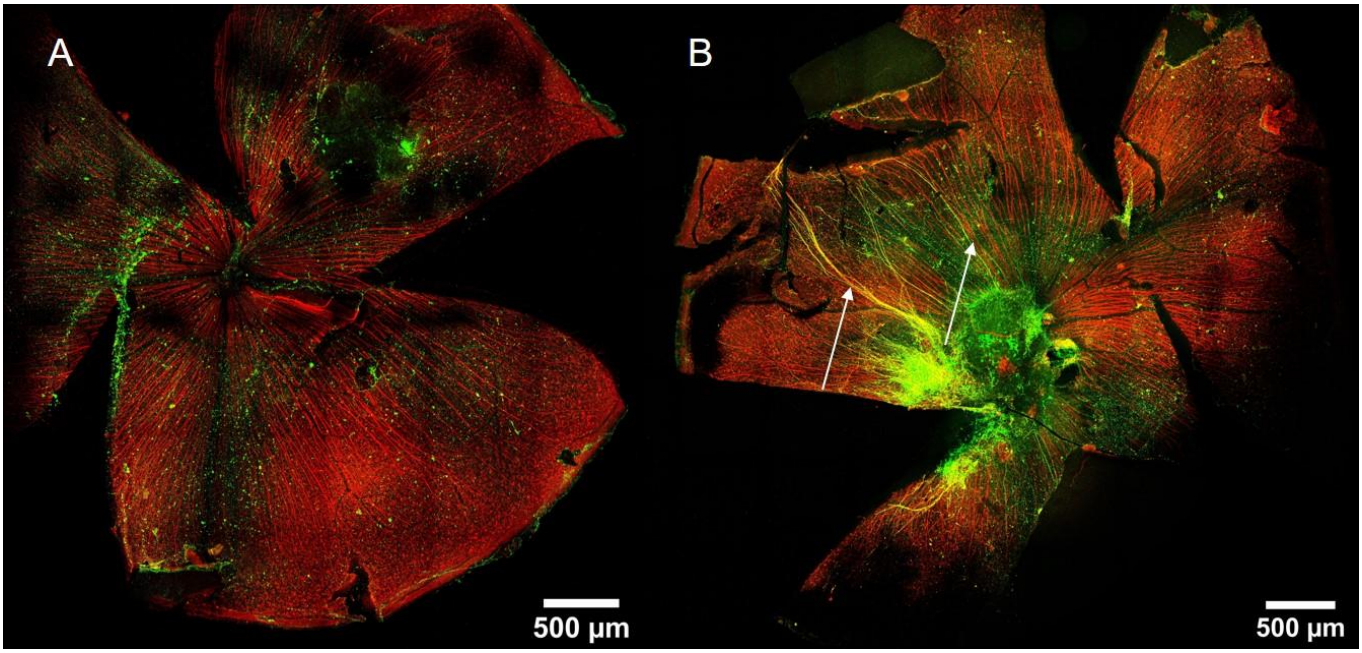



**Figure 4. Dissected eyes, two weeks post-RGC transplantation.** Thy1 was used to detect RGCs and β3-tubulin (TUJ-1) used as general neuronal marker. (A) without PODS® (B) with PODS®. Significantly improved maturation with increased levels of axon growth (arrows) was achieved in the PODS-treated eye.

## Conclusions

- PODS® nanocrystals achieve enhanced RGC phenotype with only a single application throughout a 10-14 day culture period compared to conventional growth factors applied at high concentrations five times.
- PODS® nanocrystals improve engraftment of transplanted cells with higher levels of survival and improved maturation/in-situ differentiation of implanted cells.

**For more information and a full list of our current PODS® growth factors, please visit our website www.cellgs.com.**

Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*

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