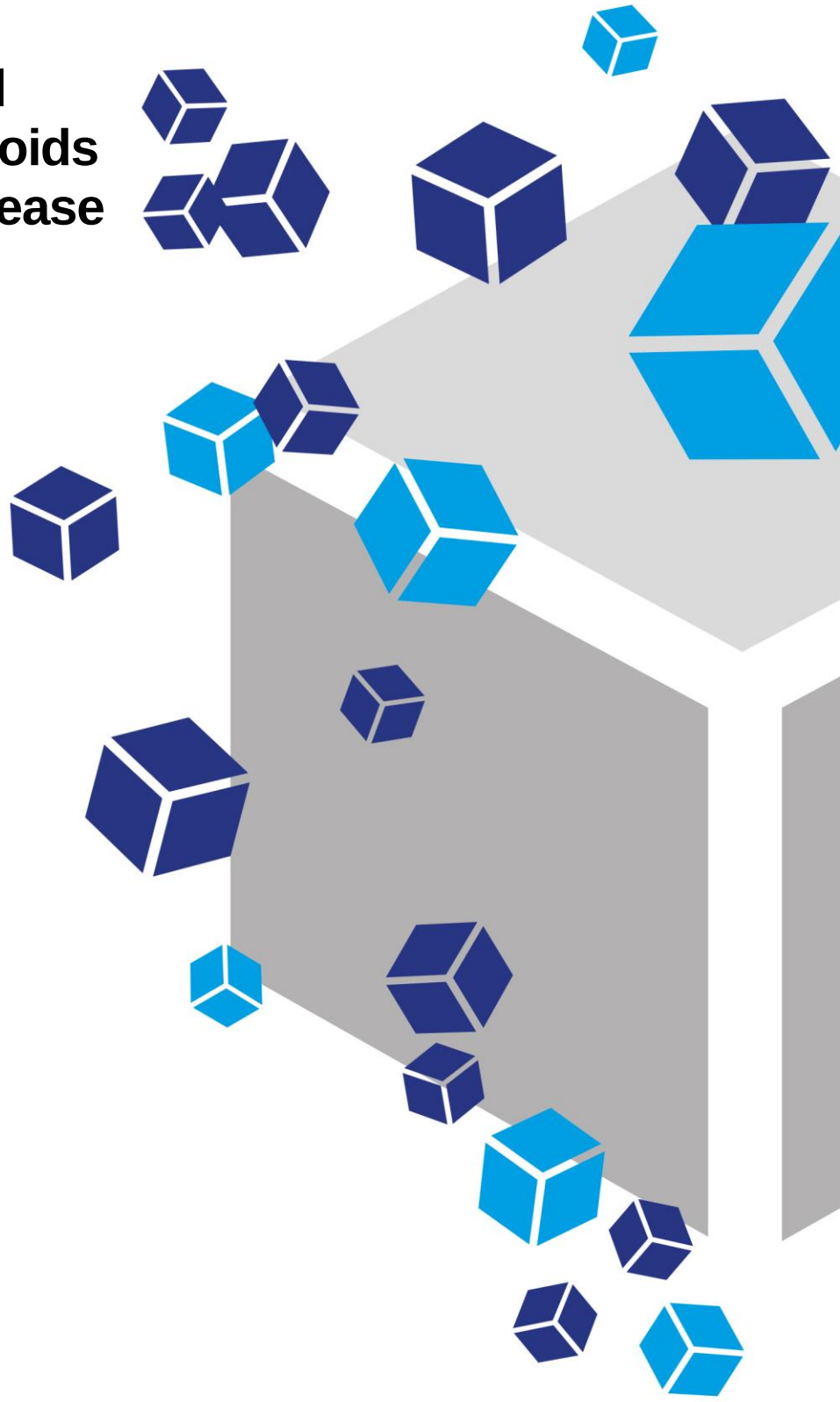
CELL guidance systems

Application Note

Improved 3D retinal ganglion cell organoids using sustained release



3D retinal ganglion cell organoid cultures utilizing PODS® BDNF and PODS® GDNF

Data Courtesy of Julia Oswald and Petr Baranov,
Harvard Medical School, Boston, MA, USA (Issued November, 5th 2018)

Introduction to PODS®

The challenge with soluble growth factors

Many proteins, especially growth factors and cytokines, when used as a reagent, degrade quickly, rapidly losing their bioactivity. This fragility hampers research and significantly limits the therapeutic potential of proteins.

Protein Micro-depots

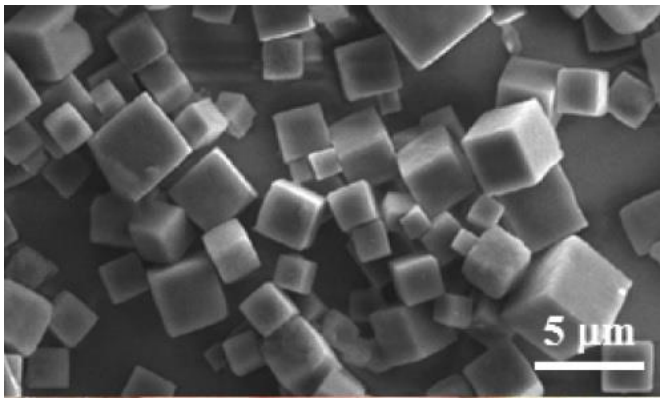
Development of a technology that can continuously replenish active protein from a local, microscopic store has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control over the growth of cells.

Introducing PODS®

PODS® technology has made the goal of a micro-depot for proteins a reality. 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.

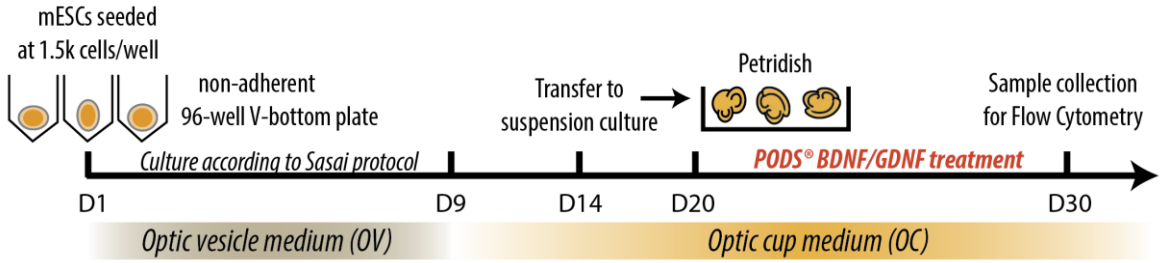


How can PODS® help my research?

PODS® are tough and will withstand physical and chemical stress, so you can handle them with ease. PODS® can be made to release intact cargo protein over days, weeks or even 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

Methods



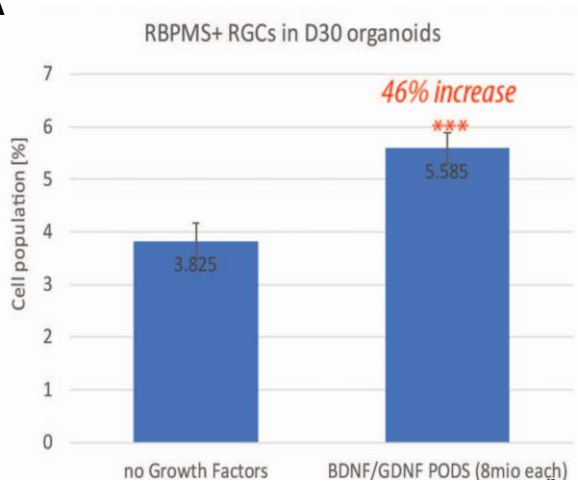
Differentiation method: For organoid formation, mouse embryonic stem cells (mESCs) were first cultured in optic vesicle medium and from day 9 transitioned to optic cup medium. PODS® growth factors were introduced to the culture system on day 20 by single addition of both PODS® BDNF and PODS® GDNF crystals. **NOTE**: During the 10-day period of PODS® growth factor treatment, only a single half media change was performed.

Existing PODS® were kept in the culture and no new PODS® were added.

Flow Cytometry: Organoids were dissociated from the petri dish and filtered through a 40 µm filter mesh. Subsequently, single cells were washed, stained with antibodies and analyzed on a MACSQuant Analyzer.

Results

A



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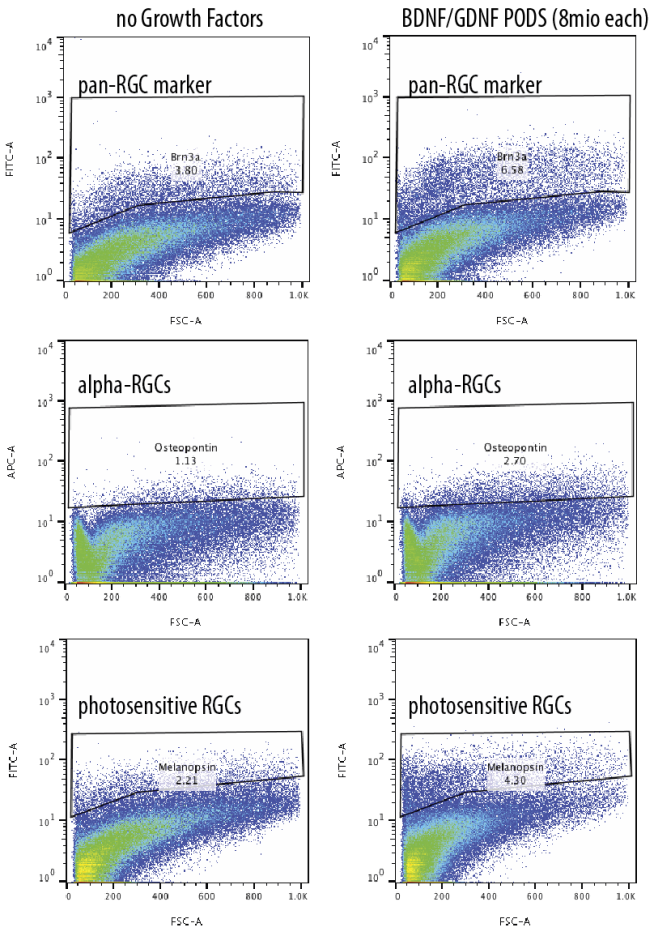

(A) Quantification of total differentiated retinal ganglion cells (RGCs) with the neuro-chemical marker RBPMS. RGC data plotted as percentage of total cell number (n=4). **(B)** Quantification of RGC sub-types. 3D-retinal organoids were cultured for 10 days either in the absence (left column) or presence of PODS® BDNF and PODS® GDNF crystals (right column). Subsequently, 3 commonly used cell markers were employed in fluorescence-activated cell sorting (FACS) to quantify the RGC sub-types produced. Analysis of FACS data shows yields of each cell subtype were increased as much as 2-fold by the addition of PODS® growth factors.

Results are based on the single addition of 8 million crystals of each PODS® BDNF and PODS® GDNF throughout a 10-day culture duration. A half medium change of basal medium was performed after 5 days without further PODS® crystal supplement. NOTE: An RGC increase approaching that achieved with PODS®, but using standard recombinant BDNF/GDNF, could only be achieved with medium supplemented with 250 ng of each GF which had to be added five times during the 10-day culture period. Organoids appeared healthier with smoother surfaces when incubated with PODS®.

Conclusions

- PODS® crystals adhere efficiently to plastic surfaces, ideal for the coating of tissue culture dishes.
- For long culture periods, a single application of PODS® crystals is effective, significantly reducing both hands-on time and cost of materials.
- PODS® achieve enhanced RGC yields compared to standard recombinant GFs with only a single addition throughout a 10-14 day culture period.
- The healthier phenotype of organoids is most likely due to reduced handling disturbance and the consistent GF levels achieved by slow release from PODS® crystals.

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*

Growth Factors
- Recombinant
- Sustained Release

Exosomes
- Purification
- Detection
- Tracking
- NTA Service

Small Molecules

Cell Counting Reagent

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- Photostable
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Gene Knock-Up System

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