A novel sustained release growth factor technology for stem cell differentiation and organoid culture

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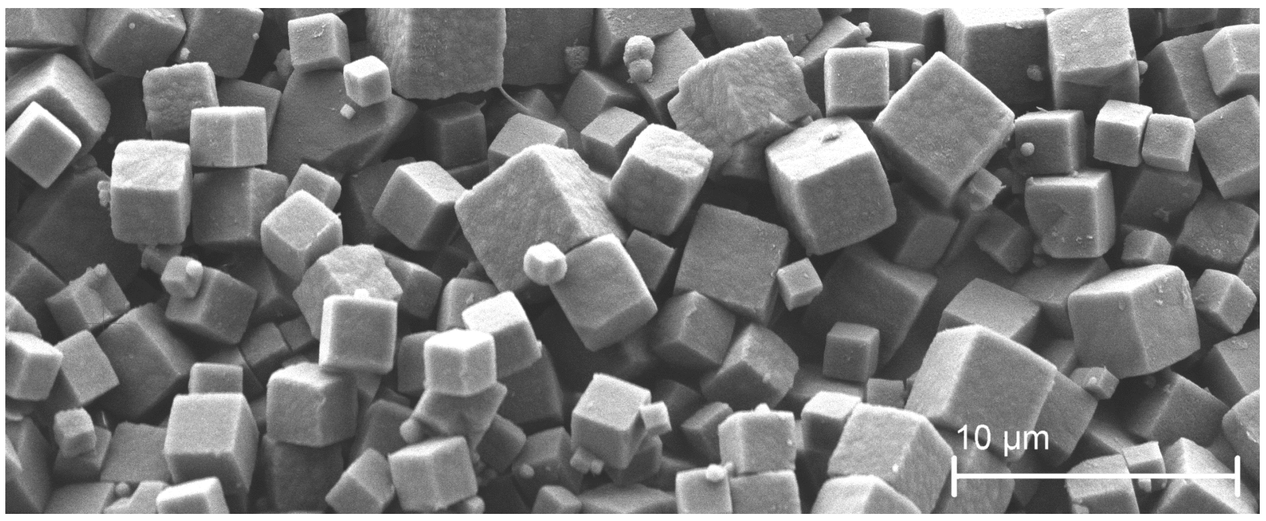
Abstract

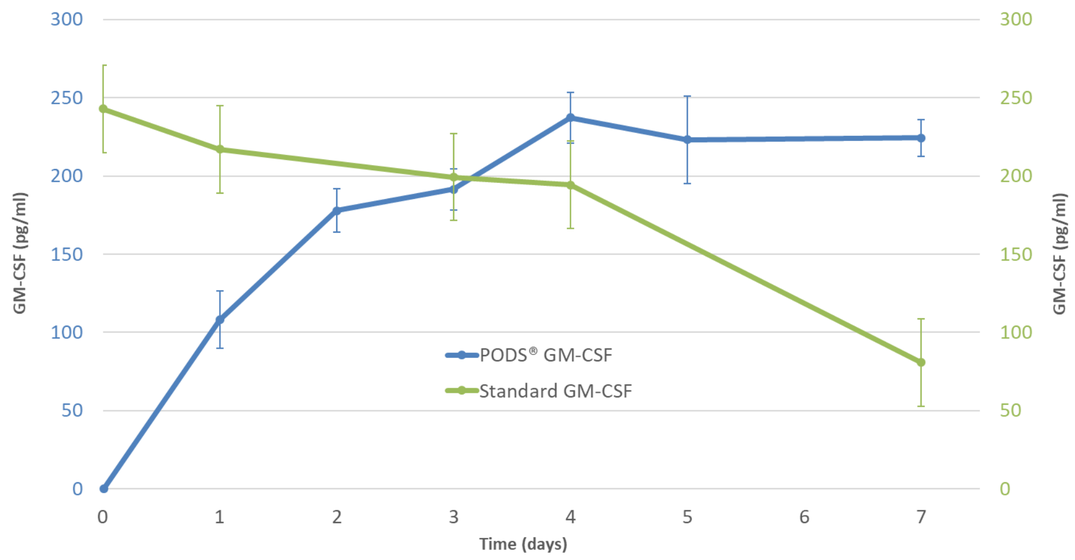
Stem cell culture and differentiation protocols are challenging and generally require a significant amount of time and reagents. One of the main issues is instability and the relatively short half-lives of growth factors (GF), which limit their use both in the lab and for transitioning into the clinic. In collaboration with Kyoto Institute of Technology, we developed a novel sustained release growth factor technology which encapsulates growth factors in a protein shell, protecting and preserving their function. PODS® (POlyhedrin Delivery System) crystals are produced in insect cells by co-expression of polyhedrin protein and a cargo protein. Highly stable, PODS® crystals degrade slowly, resulting in a steady release of cargo protein over several weeks. Here, we outline how PODS® crystals can be applied to improve 3D organoid differentiation protocol.

Introduction

PODS® crystals encase and protect the protein of interest within a polyhedrin protein shell. PODS® crystals contain intact, native and functional cargo protein. They are extremely stable in storage, highly durable in most experimental conditions, and steadily release active cargo protein by slowly degrading over several weeks. This sustained release mechanism of PODS® growth factors can be used in many ways, e.g. to cultivate organoid cultures, which we demonstrate in this poster.



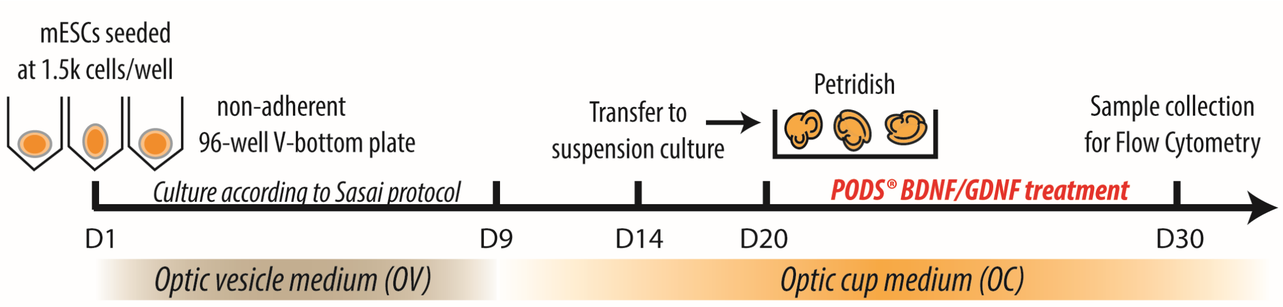
Sustained release of growth factor from PODS®



Release from PODS® GM-CSF crystals and GM-CSF stability, quantified by ELISA. ATDC5 cells in DMEM + 10% FBS were incubated at 37°C for 7 days with either PODS® GM-CSF (blue trace) or standard soluble GM-CSF (green trace). Media was removed at indicated time points. Error bars represent 3 technical repeats.

Improved 3D retinal ganglion cell (RGC) organoids using PODS® crystals

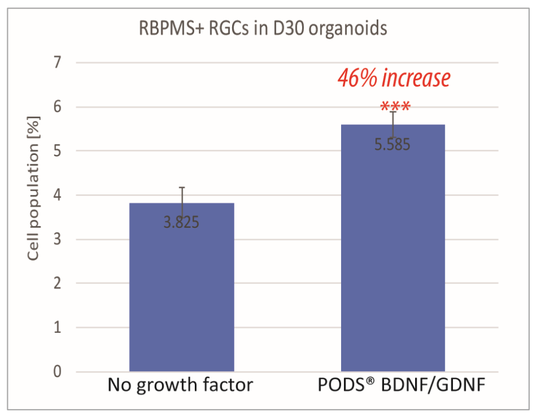
Methods



For organoid formation, mouse embryonic stem cells (mESCs) were first cultured in optic vesicle medium and from day 9 transitioned to optic cup medium. On day 20, PODS® growth factors were introduced to the culture system for a further 10 days. **During the 10-day period of PODS® GF treatment, only a single half-media change was performed without any addition of PODS® crystals.**

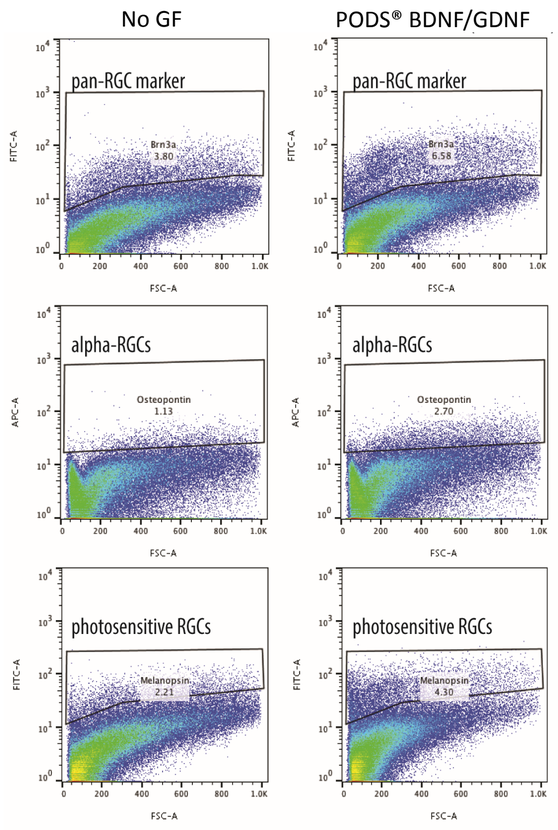
3D-retinal organoids were cultured in 3 different conditions: No GF, standard recombinant GF or with PODS® BDNF and PODS® GDNF crystals. Subsequently, 3 fluorescence-activated cell sorting (FACS) was used to quantify the RGC sub-types produced using 3 commonly-use cell markers.

Results



Quantification of total differentiated RGCs with the neuro-chemical marker RBPMS.

FACS analysis shows 46% increase RPMS + RGCs with PODS® crystals compare to no GF. Furthermore, RGC yields of each RGC subtype were increased by as much as 2.3-fold by the addition of PODS® growth factors.



Quantification of RGC sub-types by FACS.

Conclusions

- **An RGC increase, approaching that achieved with PODS®, could only be attained by supplementing with 250 ng each of standard BDNF/GDNF, which was added every two days over the 10 day culture period. Additionally, a healthier phenotype of organoids was achieved, most likely due to reduced handling disturbance and the consistent growth factor levels achieved by the sustained release from PODS® crystals.**
- PODS® crystals adhere efficiently to plastic surfaces, ideal for 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.
- Compared to standard soluble GFs, using PODS® crystals results in similar RGC yields but a healthier phenotype of RGC organoids.