PODS® nanodevices: armored growth factors transition seamlessly from 2D and 3D cultures to *in-vivo* use

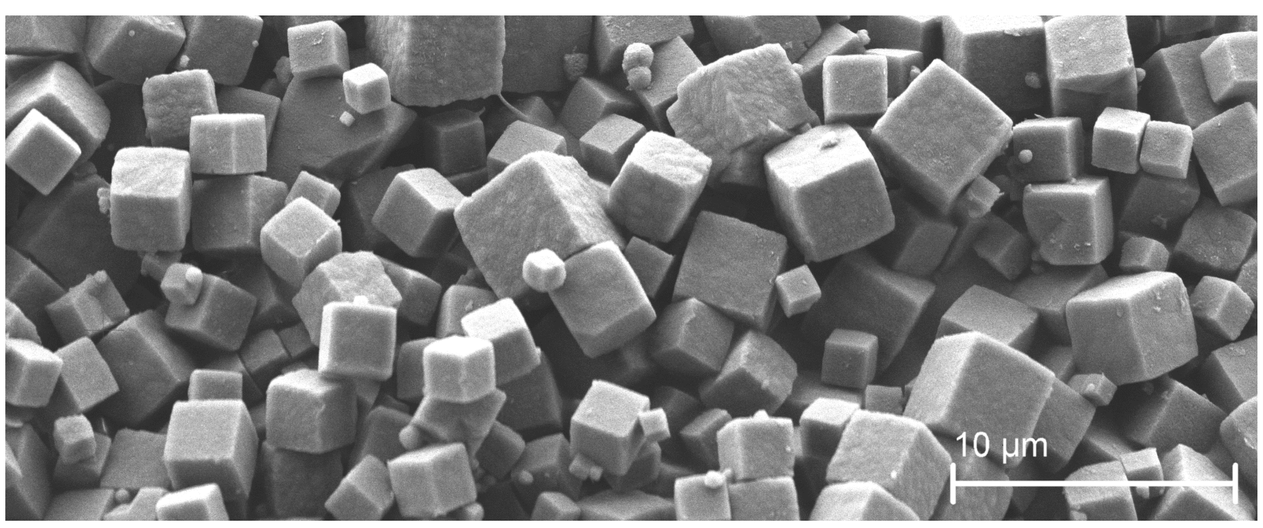
Christian Pernstich*, Julia Oswald, Petr Baranov, Hajime Mori and Michael Jones
*Cell Guidance Systems, United Kingdom (CP, MJ); Harvard Medical School, USA (JO, PB) and Kyoto Institute of Technology, Japan (HM); *chris.pernstich@cellgs.com*

Abstract

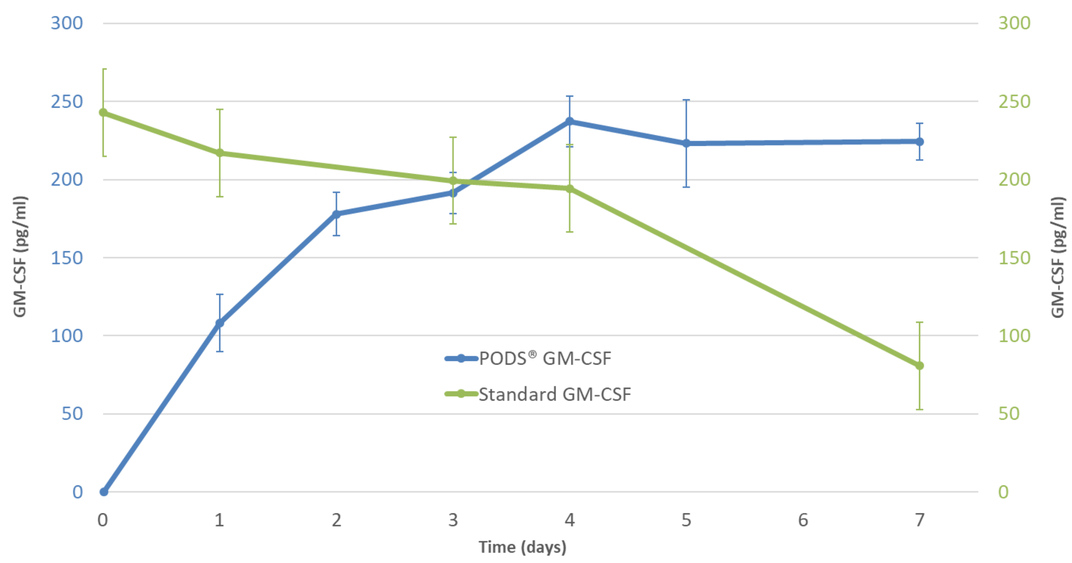
Stem cell culture and differentiation protocols are challenging and generally require a significant amount of time and reagents. One of the main issues is the instability and relatively short half-live of growth factors, which limit their use both in the lab and for transitioning into the clinic. In collaboration with Kyoto Institute of Technology, we developed a novel sustained-release technology which encapsulates growth factors in a protein shell, protecting and preserving their function. PODS® (POlyhedrin Delivery System) nanodevices are highly stable and degrade slowly, resulting in a steady release of cargo protein over several weeks. A true platform technology, PODS® nanodevices can be applied in different culture systems, *in-vitro* as well as *in-vivo*.

Introduction

Crystalline PODS® nanodevices encase and protect the protein of interest within a polyhedrin protein shell. Coexpressed in insect cells, 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, long-term *in-vitro cultures* as well as *in-vivo* for therapeutic protein delivery.



Sustained release from PODS® nanodevices

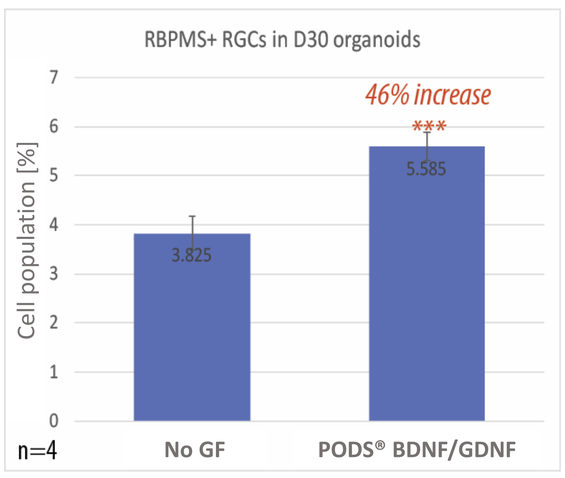


Release from PODS® GM-CSF crystals and GM-CSF stability, quantified by ELISA. ATDC5 cells in DMEM + 10% FBS were incubated at 37°C for 7 days with either PODS® GM-CSF (blue trace) or standard soluble GM-CSF (green trace). Media was removed at indicated time points. Error bars represent 3 technical repeats.

In-vitro and *in-vivo* case studies

PODS® nanodevices in retinal ganglion organoid cultures

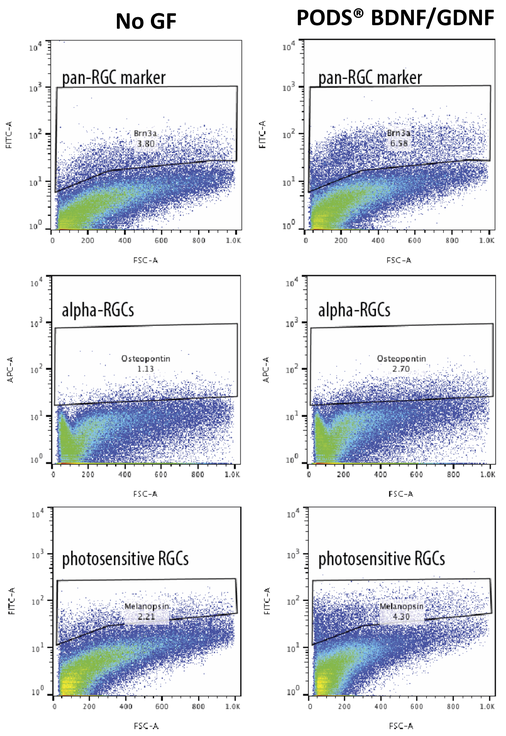
A



(A) Quantification of total RGCs with the neuro-chemical marker RBPMS. RGC data plotted as percentage of total cell number.

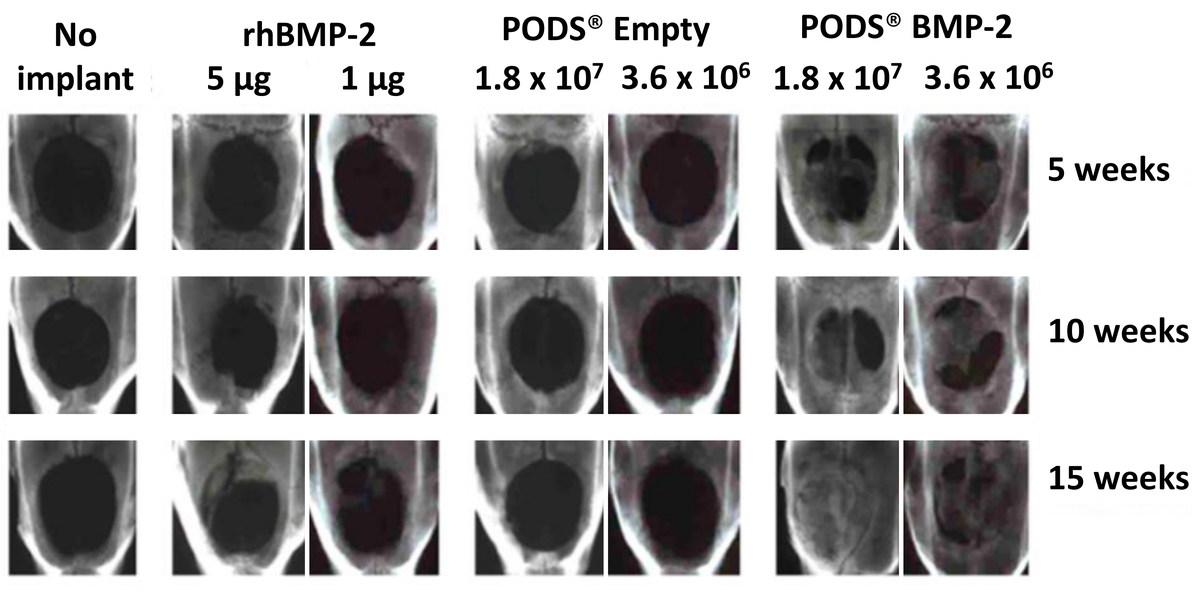
(B) Quantification of RGC sub-types. Retinal organoids were cultured for 10 days either in the absence (left column) or presence of PODS® BDNF and PODS® GDNF crystals (right column). RGC sub-types were quantified using

B



fluorescence-activated cell sorting (FACS). Analysis shows yields of each cell subtype were increased as much as 2.4-fold by the addition of PODS® growth factors.

PODS® nanodevices in bone regeneration in rat calvaria



PODS® BMP-2, incorporated into absorbable collagen sponges, at low and high doses produced significantly more bone at all time points compared with rhBMP-2 or PODS® Empty. Histological analysis revealed that crystals were still visible at around 12 weeks. No inflammatory response, adverse immune reaction or side-effects such as ossification was observed.

Reference:

- Matsumoto et al. Bone regeneration by polyhedral microcrystals from silkworm virus. Scientific reports 2012; 2:935

Conclusions

- For long culture periods, a single application of PODS® nanodevices is effective, significantly reducing both hands-on time and cost of materials.
- PODS® nanodevices deliver on long-term sustained release, by locking growth factors in a highly stabilized form.
- PODS® nanodevices protect cargo protein to generate high levels of efficacy from low, non-toxic doses.
- PODS® nanodevices can be utilized to pattern surfaces or can be incorporated into biomaterials, making it easy to functionalize scaffolds.