Armoured growth factors for tissue engineering

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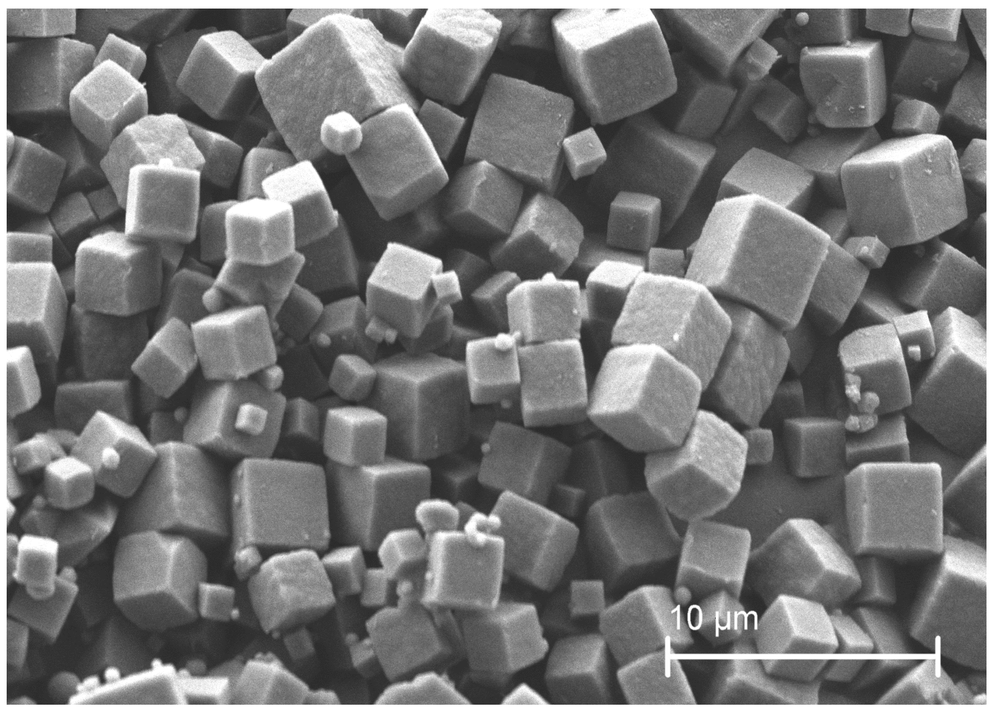
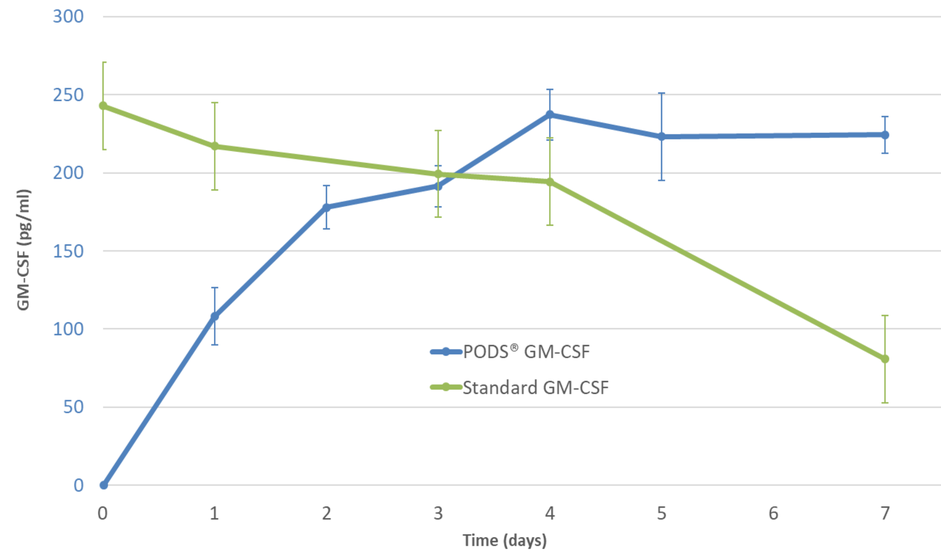
Introduction

The inherent instability of recombinant growth factors (GFs), with typical half-lives ranging from minutes to hours, limits their utility in the lab and the clinic. Efficacy may be improved by encapsulation within biomaterials to provide sustained bioavailability over a longer time. However, current delivery systems are limited by protein denaturation and burst release. Refinement of delivery systems to provide sustained release and improved retention may provide therapeutic efficacy at lower doses, improving cost-effectiveness and preventing adverse side effects. PODS (POlyhedrin Delivery System) is a highly durable, crystalline product which encases a GF of interest within polyhedrin protein. The stability of PODS means that crystals degrade slowly, resulting in a steady release of intact, native and functional cargo protein, over several weeks at physiologically-relevant levels.



PODS visualized by scanning electron microscopy (SEM)

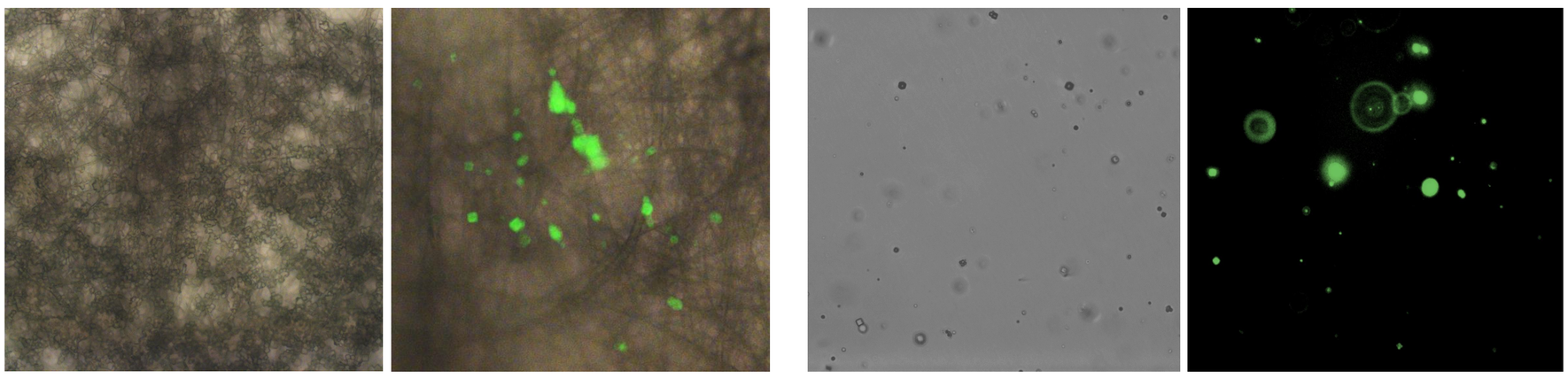
Sustained release of growth factor from PODS



Release from PODS GM-CSF and GM-CSF stability, quantified by ELISA. ATDC5 cells in DMEM + 10% FBS were incubated at 37°C for 7 days with $5x10^5$ PODS GM-CSF (blue) or 250 pg/ml standard soluble GM-CSF (green). GM-CSF was measured by ELISA. N=3

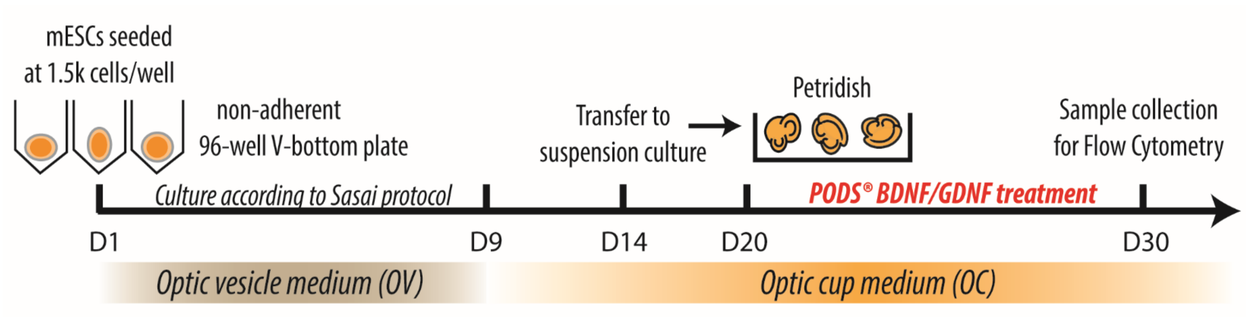
Functionalising biomaterials with PODS

PLA + PODS Empty | PCL + PODS GFP | Hydrogel (RADA-16) + PODS GFP



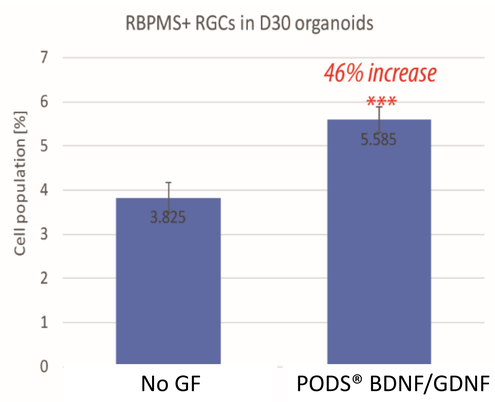
PODS™ can be incorporated into materials such as hydrogels, and poly(ε-caprolactone) (PCL) or poly(DL-lactide) (PLA) electrospun fibre sheets. Fluorescence microscopy of PODS™ GFP incorporated into PCL or hydrogels indicates that PODS cargo activity is unaffected by incorporation.

Improved 3D retinal ganglion cell organoids using PODS BDNF and PODS GDNF



For organoid formation, mouse embryonic stem cells (mESCs) were 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 PODS® treatment, only a single half-media change was performed without any additional PODS® crystals.**

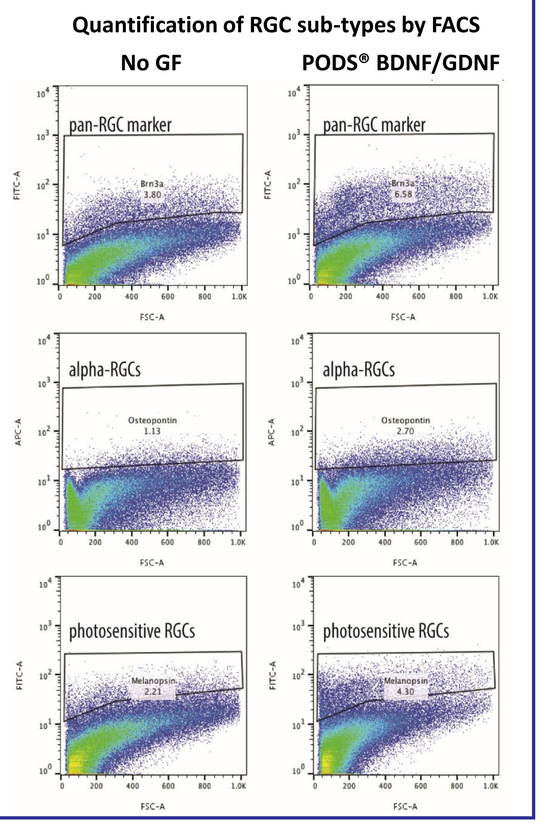
3D-retinal organoids were cultured with no GF, standard GF or PODS® BDNF and PODS® GDNF. The 3D retinal ganglion cell (RGC) sub-types produced were quantified by flow cytometry using three cell markers.



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

FACS indicates a 46% increase in RPMS + RGCs with PODS® crystals, compare to no GF. RGC yields of each subtype increased by up to 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, 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