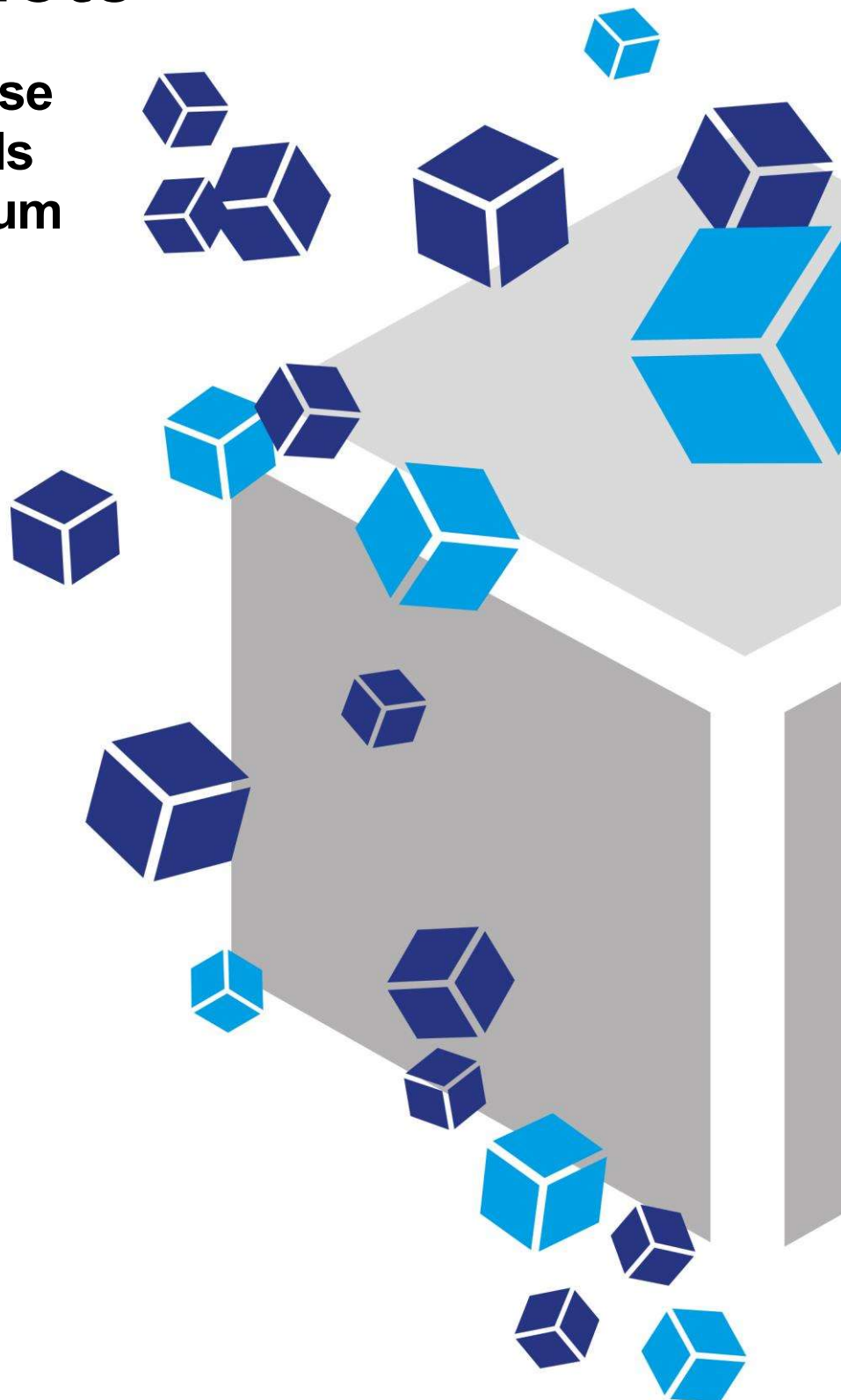


Technical Note

**Growth factor release
from PODS[®] crystals
in serum-free medium**



Growth factor release from PODS[®] crystals in serum-free medium

Introduction to PODS[®]

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

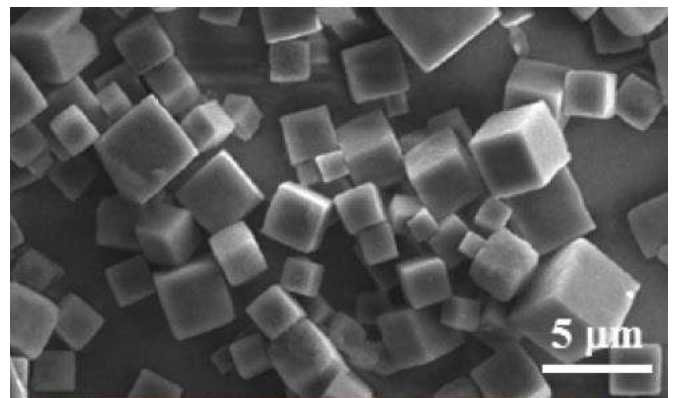
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

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

PODS[®] are microcrystalline depots which can release a constant supply of bioactive growth factors to cells. This process is dynamic and requires the presence of proteases to degrade the protein crystal lattice to release the growth factor. The proteases can originate from the cells (that need the growth factor) or can be derived from components of the cell culture system, such as added serum or matrix protein coatings e.g. collagen. Specifically, matrix metalloproteinases have been found to release protein cargo from PODS[®] crystals¹.

To measure the effect of cell-generated proteases on the release of PODS[®] cargo growth factors, serum-free conditioned media, containing proteases released from cell lines, was incubated with PODS[®] crystals. ELISA was used to measure the amount of free growth factor released. We demonstrate that the majority of cell lines tested achieve sufficient release of growth factor to generate a biological effect. The proliferative effect exceeded that achieved by conventional growth factors even when used at high levels.

Methods

Serum-free conditioned medium production

- 1) 4.2×10^5 cells were seeded in 14 mL of complete medium in a T-75 tissue culture flask.
- 2) Flasks were incubated at 37°C for 24 hours to facilitate cell attachment.
 - a. **Adherent cell lines:** medium was removed and cells washed in PBS to remove any trace serum.
 - b. **Suspension cell lines:** cells were centrifuged at 300 x g and subsequently washed in PBS to remove any trace serum. Cells were then centrifuged a second time before being transferred back to the original flask.
- 3) 14 mL fresh serum-free medium was added to the flasks and returned to the incubator.
- 4) Cells were cultured for 2 days to condition the medium with cell-secreted proteases.
- 5) Medium was harvested and passed through a 0.2 µm filter to remove residual cells and debris.

Release from PODS[®] in serum-free conditioned medium

1. 96 well plates were prepared as described in '[Creating PODS[®] crystal monolayers on multi-well tissue culture plates](#)'. Briefly:
 - a. PODS[®] stock suspensions were diluted in sterile PBS in a biosafety cabinet.
 - b. 100 µL of each PODS[®] suspension was pipetted into wells of a 96 well plate.
 - c. Plates were centrifuged at 3000 x g for 20 minutes using a plate-holding rotor.
 - d. PBS was removed and the plates dried for 1 hour in a biosafety cabinet.
2. After drying, 100 µL of either complete medium with 10% fetal bovine serum, or serum-free conditioned medium was added to wells containing PODS[®] crystals, as well as to empty wells to serve as blanks.
3. Plates were incubated at 37°C, 5% CO₂ for 4 days. Growth factor levels were then measured in the medium using ELISA.

NIH/3T3 cell culture: PODS[®] FGF-2

1. 24 well plates were prepared as described in '[Creating PODS[®] crystal monolayers on multi-well tissue culture plates](#)'.
2. After drying, fibroblast cells were added in complete medium and allowed to adhere for 24 hours.
3. Complete medium was removed, and exchanged with 500 µL serum-free medium, either unsupplemented, or containing conventional FGF-2.
4. Plates were incubated at 37°C, 5% CO₂ for 3 days. Cell numbers were then assessed using a colorimetric cell counting assay ([Orangu™](#), Cell Guidance Systems).

Results

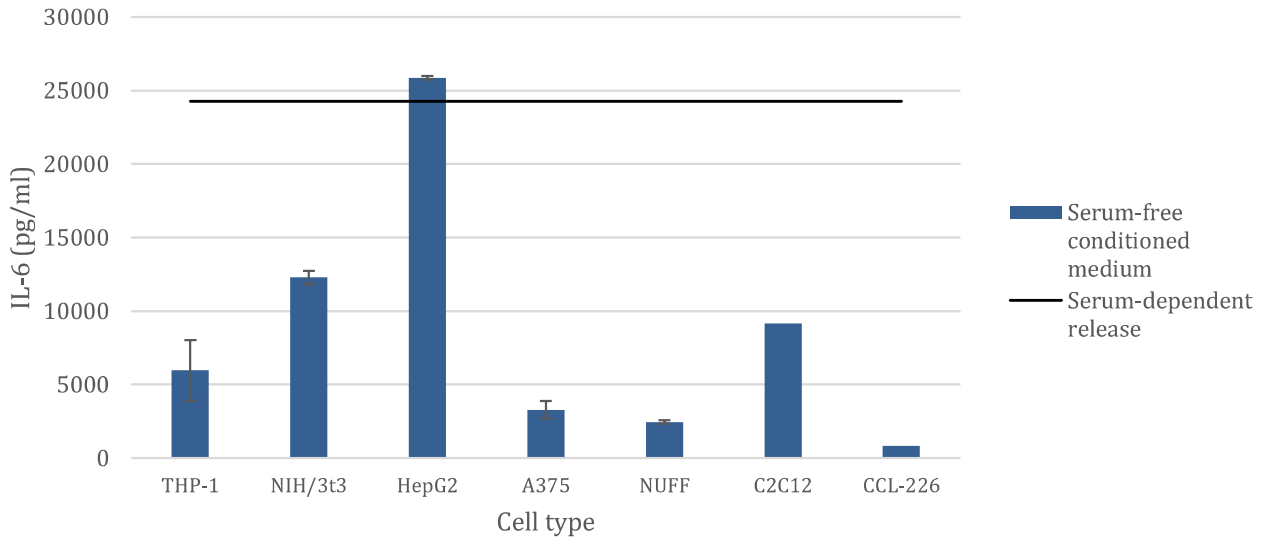


Figure 1. Release of IL-6 from PODS® IL-6 crystals. Concentrations were measured at day 4 by ELISA. Serum-dependent release determined by mean measurements of each cell line’s complete medium, standard deviation ± 2630 pg/ml ($\pm 10.84\%$). Error bars represent standard deviation calculated from at least 3 technical repeats.

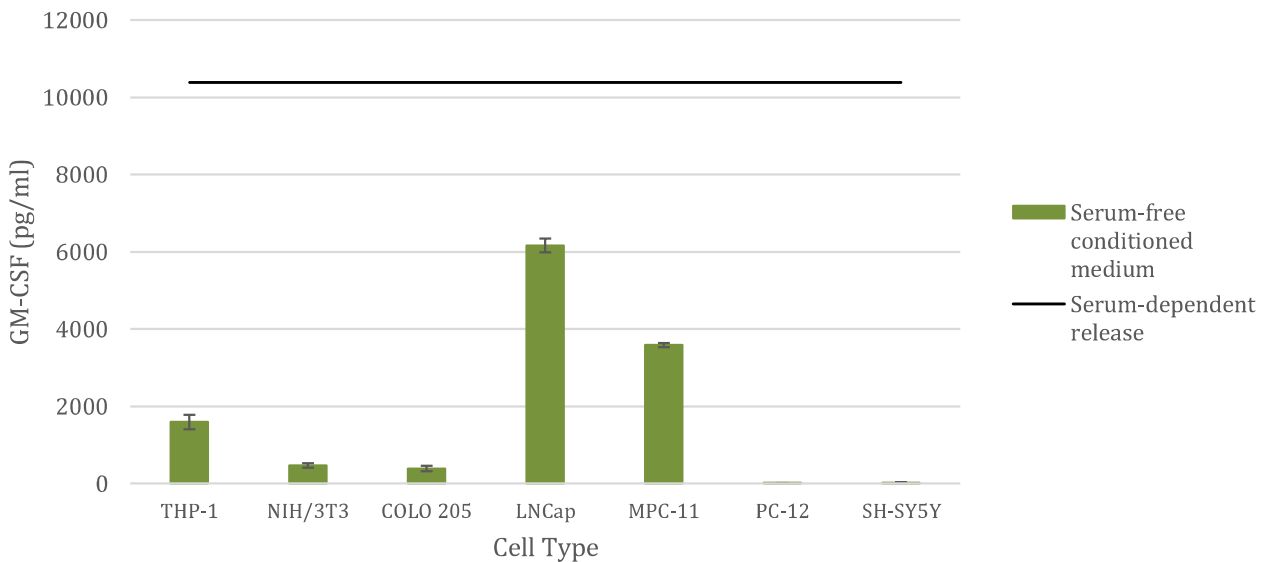


Figure 2. Release of GM-CSF from PODS® GM-CSF crystals. Concentrations were measured at day 4 by ELISA. Serum-dependent release determined by mean measurements of each cell lines complete medium, standard deviation ± 145 pg/ml ($\pm 1.4\%$). Error bars represent standard deviation calculated from at least 3 technical repeats.

In order to demonstrate that levels of growth factor released from PODS[®] have similar biological effects when used for in vitro experiments, the effect of a single dose of PODS[®] FGF-2 incubated with fibroblast cells in a serum-free culture was compared to that of conventional FGF-2 (Figure 3).

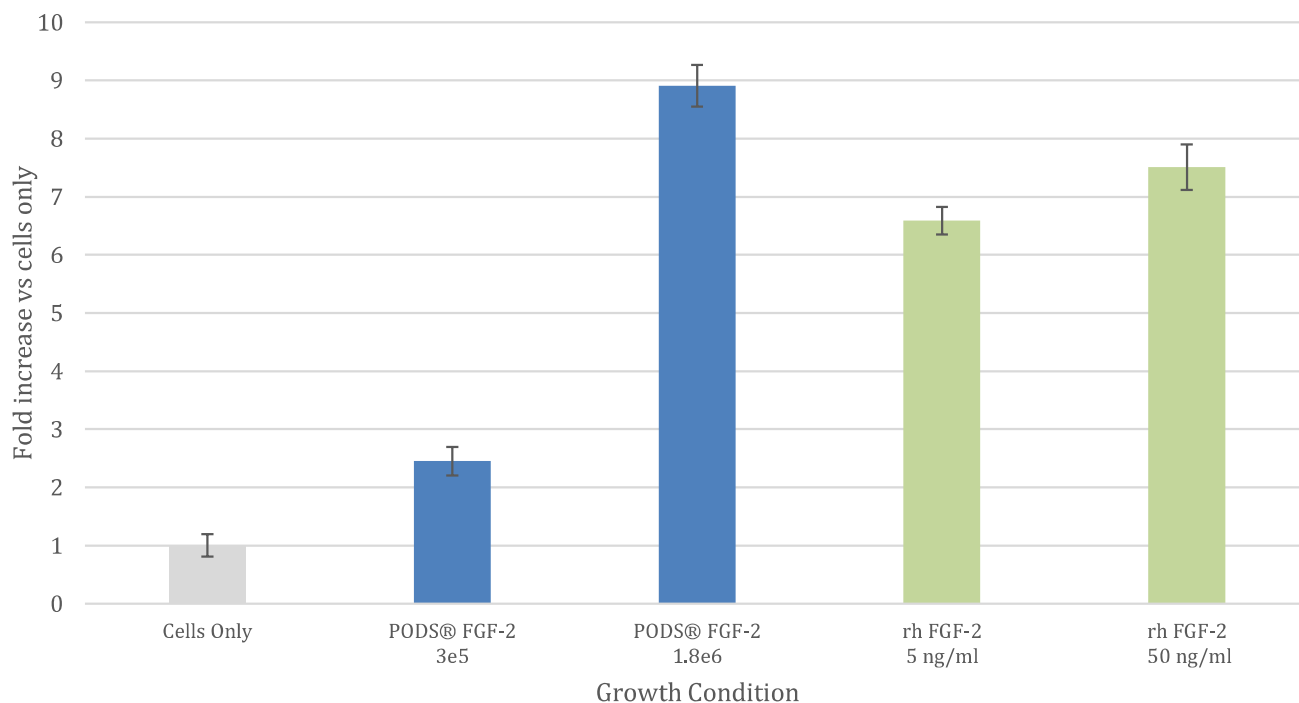


Figure 3. Proliferation of NIH/3t3 cells in serum-free media conditions, cultured over 3 days. Cell growth was stimulated by low and high PODS[®] FGF-2 amounts, with the higher amount exceeding the effect of typical quantities of conventional FGF-2. Cell numbers for all conditions were quantified using a colorimetric cell counting assay (Orangu[™]) and normalized to the value from the “cells only” measurement. Error bars represent standard deviation calculated from at least 3 technical repeats. 3×10^5 and 1.8×10^6 PODS[®] per well are equivalent to 1.5×10^5 and 9.5×10^5 PODS[®]/cm² respectively.

Conclusions

- Release of cargo from PODS[®] growth factors can be stimulated by a serum-containing complete medium.
- Release of cargo from PODS[®] growth factors are stimulated by cell secreted proteases in the absence of serum.
- Growth factors released from PODS[®] in serum free culture can out-perform conventional growth factors

Reference

- (1) Matsuzaki, Y.; Maruta, R.; Takaki, K.; Kotani, E.; Kato, Y.; Yoshimura, R.; Endo, Y.; Whitty, C.; Pernstich, C.; Gandhi, R.; Jones, M.; Mori, H. Sustained Neurotrophin Release from Protein Nanoparticles Mediated by Matrix Metalloproteinases Induces the Alignment and Differentiation of Nerve Cells. *Biomolecules* **2019**, 9 (10). <https://doi.org/10.3390/biom9100510>.

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

Growth Factors

- Conventional (unformulated)
- PODS® - Sustained release

Exosomes

- Exo-spin™ - Purification
- ExoLISA™ - ELISA-like detection
- Instant Exosomes™ - purified and characterized
- NTA Service
- Freeze drying service

PeptiGel®

- Tunable self-assembling peptide hydrogels

Other products and services

- Small Molecules
- Softwell™ - 2D hydrogel (Europe only)
- Orangu™ - Cell counting reagent
- LipoQ™ - Lipid quantification assay
- Primary Hepatocytes

Cytogenetics

- Karyotype Analysis
- Array Hybridization

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