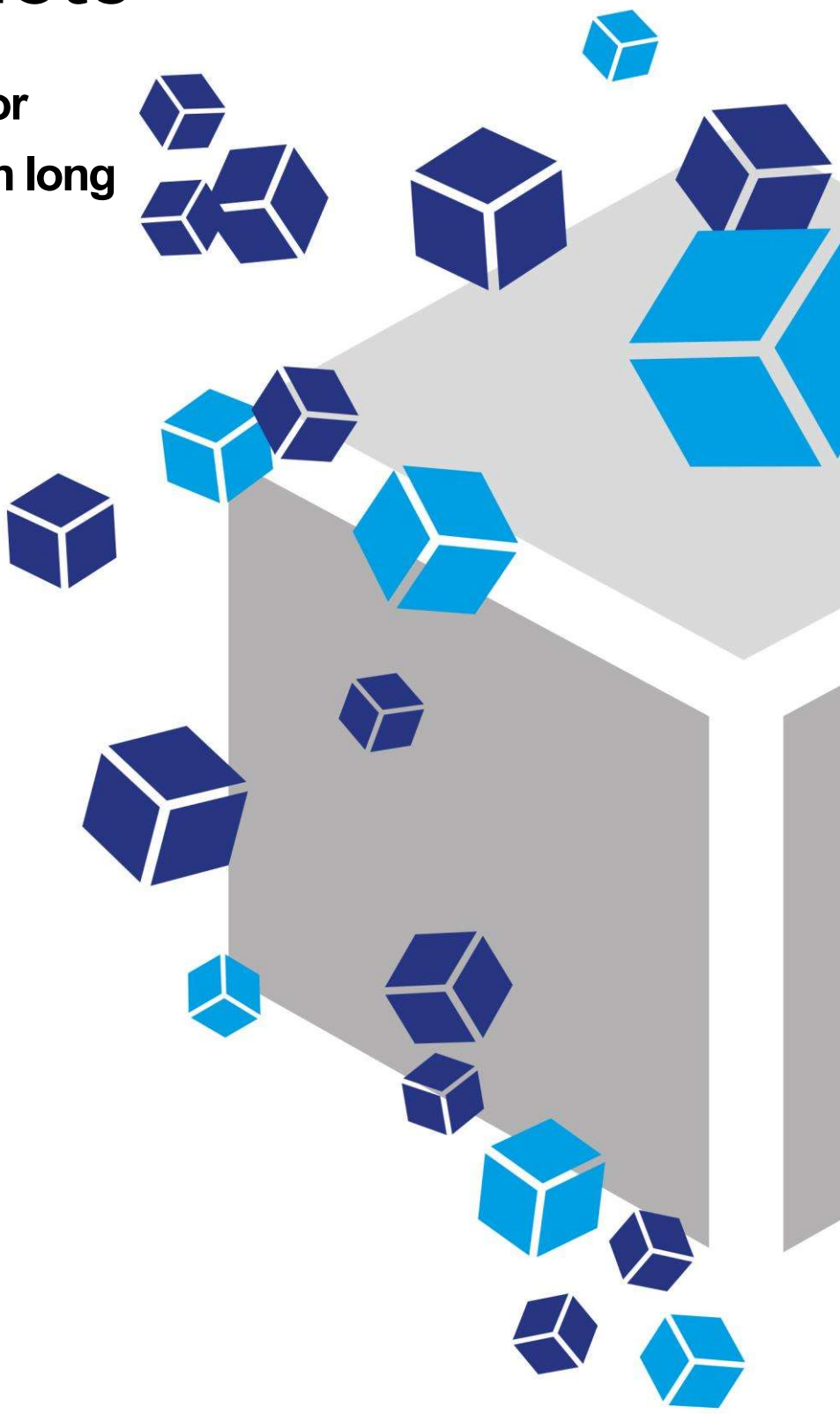
# Technical Note

## Sustained growth factor release using PODS® in long term cell culture



# Sustained growth factor release using PODS® in long term cell culture

## 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

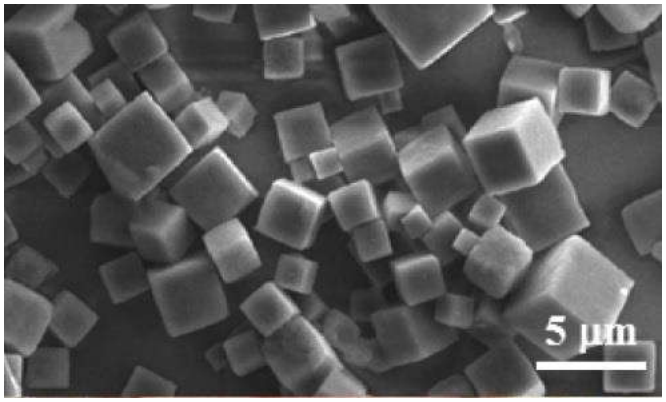
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control over the growth of cells.

### 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

# Overview

Conventional soluble growth factors, when used in cell culture, must typically be replenished multiple times a week due to the instability of these molecules. With growth factors often making up the majority of the cost of a medium formulation, this leads to a considerable cost burden, as well as increased handling frequency. A further consideration is the effect that cycles in growth factor concentrations with large amplitudes can have on cells (Fig.1). Excess amounts of growth factors are typically added to compensate for the rapid decay of the molecules. Cycling between excess and insufficient growth factor availability causes cellular stress resulting in experimental variation.

The data presented here demonstrates the ability of PODS® crystals to provide sustained levels of growth factor from a single dose, with detectable growth factor available for at least 9 weeks following initial application. With PODS®, media exchanges can be reduced to once a week in long term experiments without requiring growth factor replacement. Furthermore, including hydrogels in 3D culture systems can modulate growth factor release rates; when PODS® are in contact with both collagen and alginate gels, high growth factor levels are sustained for a longer period of time than with media only.

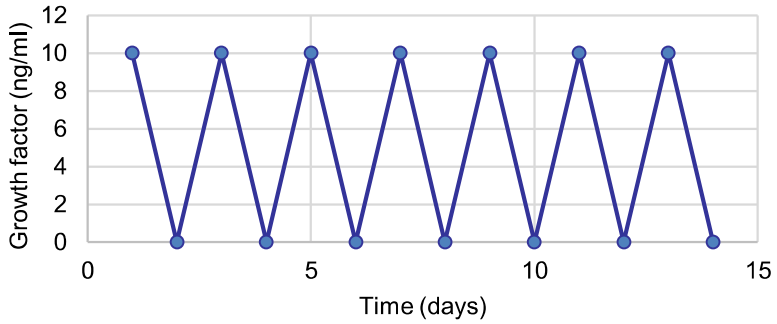



**Figure 1. Schematic depiction of cyclic increases and decreases in growth factor concentration in culture. The speed of degradation is dependent on the specific growth factor and other components of the culture system. The above diagram illustrates FGF2 levels in serum free culture when measured immediately following fresh FGF2 addition and on alternative days.**

# Methods

**Adhering PODS® crystals to tissue culture (TC) multi-well plates or inserts:** PODS® crystals were dried onto the surface of tissue culture plates using the method described in another Technical Note. Briefly, dense layers of either PODS® IL-06 (catalogue number PPH10) or PODS® GM-CSF (catalogue number PPH8) containing 1 × $10^6$ PODS®/well (3.125  1 × $10^6$ PODS®/$cm^2$) were created by first pipetting 100 µl of PODS® solution into each well of a 96-well plate, then centrifuging for 20 minutes at 3000 × g. The supernatant was then removed and the crystals left to dry on to the plate before adding media and/or gel.

**Gel casting onto a PODS® crystal layer:** Gels were prepared according to the manufacturers' instructions and cast into the wells of the TC multi-well plate above the dried PODS® crystal monolayer.

For 3% alginate gel, the required amount of powdered sodium alginate was weighed out and sterilized with UV light for 1 hour, then dissolved in sterile distilled water. 50 µl of gel was dispensed into each well of a 96-well TC plate containing PODS® monolayers. The gel was crosslinked with an equal volume of 150 mM $CaCl_2$ for 30 min, which was then removed and 100 µl culture medium containing 10% FBS was added.

Collagen Type I-A gels (Cellmatrix® Collagen Type-I-A, FUJIFILM) were prepared on ice according to the manufacturer's instructions as above. 50 µl of gel was dispensed into each well. Following casting, the plate was incubated at 37°C to allow the gel to solidify, then 100 µl culture medium containing 10% FBS was added. Half or full medium changes were performed once a week for the duration of the experiment.

**Analysis:** Following collection of samples for analysis, ELISA assays (SEKB10395, SEK10015, SinoBiological) were performed to measure the levels of growth factor in the supernatant.

# Results

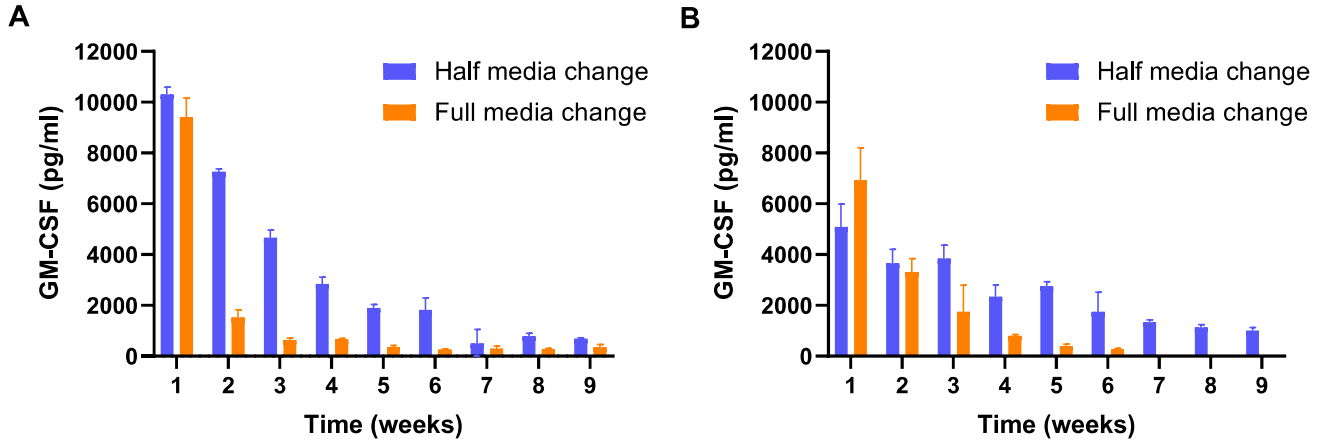



**Figure 2. Release of GM-CSF over 9 weeks from PODS® GM-CSF crystal monolayer with (A) no gel, or (B) underneath collagen gel incubated with serum-containing media, quantified by ELISA.** PODS® GM-CSF crystals (1 x $10^6$) were dried onto 96-well plates. Subsequently, (A) RPMI + 10% FBS was added to each well or (B) collagen gel was cast on top of the crystal layer and DMEM + 10% FBS was added. the plate was then incubated at 37°C. Medium was removed at indicated time points. GM-CSF was quantified by ELISA. Error bars represent standard deviation calculated from at least 3 technical repeats.

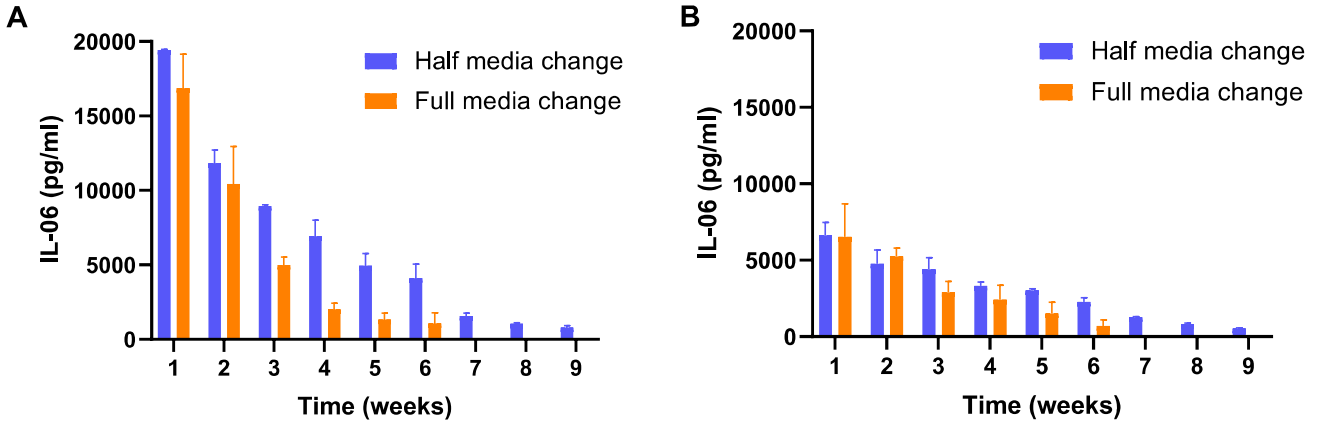



**Figure 3. Release of IL-06 over 9 weeks from PODS® IL-06 crystals (A) underneath collagen and (B) alginate gel, incubated with serum-containing media, quantified by ELISA.** PODS® IL-06 crystals (1 x $10^6$) were dried onto 96-well plates, then gels of either (A) Type I collagen or (B) 3% alginate were cast on top of the crystal layer. Subsequently, DMEM + 10% FBS was added to each well and incubated at 37°C. Medium was removed at indicated time points. IL-06 was quantified by ELISA. Error bars represent standard deviation calculated from at least 3 technical repeats.

# Conclusions

- For long term culture, a single application of PODS® crystals provides a sustained supply of growth factors, significantly reducing both hands-on time and cost of materials.
- Adding a hydrogel into the culture system can slow the release rate from PODS® and maintain a higher level of growth factor compared with no gel, particularly when half media changes are used.
- Half media changes maintain high growth factor concentrations for a longer period of time than full media changes.
- Using PODS® gives a smoother supply of growth factor over time compared to the fluctuations normally experienced in culture.
- PODS® growth factors release cargo from multiple gel types.

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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