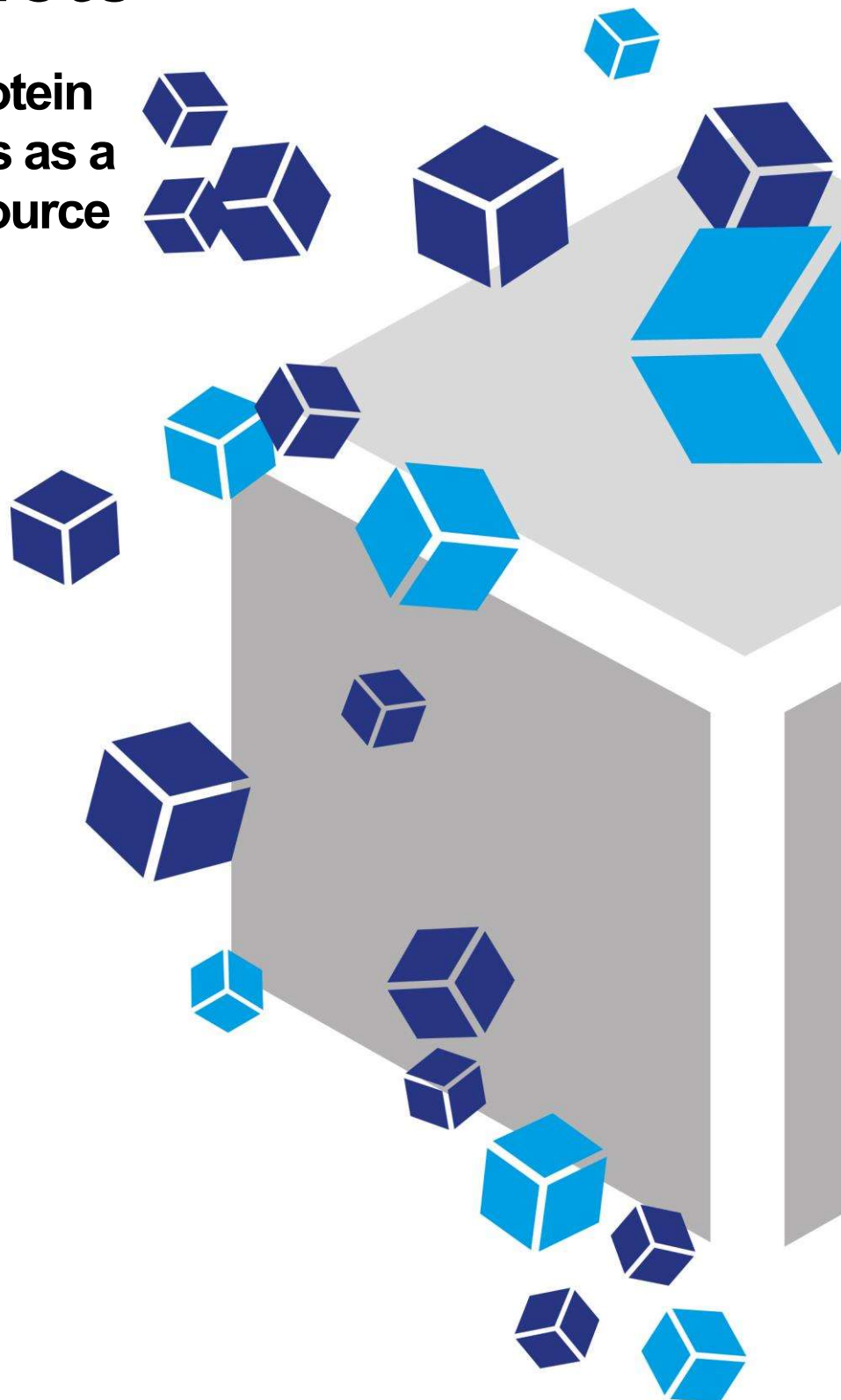


Technical Note

**Release of cargo protein
from PODS[®] crystals as a
function of serum source
and concentration**



Release of cargo protein from PODS[®] crystals as a function of serum source and concentration

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

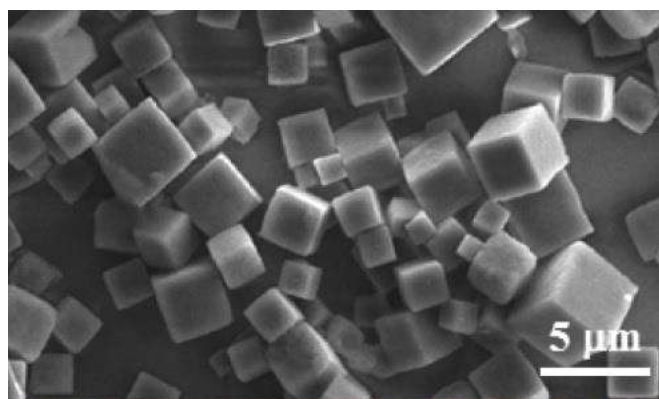
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

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

Serum is a key component for growing and maintaining many cell lines, but the growth requirements vary between cell types and optimal serum conditions need to be adjusted accordingly. Proteases present in cell culture medium aids the release of growth factors from PODS[®] crystals, by slowly degrading the polyhedrin protein crystal lattice.

The following experiments show the release of IL-6 and GM-CSF from PODS[®] crystals, respectively, in a cell-free environment with a dose-range (0% to 10%) of four standard serum types: sterile filtered fetal bovine serum (FBS), heat inactivated FBS, bovine calf serum (BCS) and horse serum (HS). ELISA was used to measure the amount of growth factor that was released from the PODS[®] co-crystals into the serum-containing medium. We demonstrate a serum dose-dependent release of cargo from PODS[®] crystals with all four tested types of serum.

Methods

PODS[®] GM-CSF and PODS[®] IL-6 (5×10^5 PODS[®]/well) were centrifuged onto 96-well plates (20 min at 3000 x g) and dried on, to generate uniformly distributed monolayer of PODS[®] crystals (more information on creating even PODS[®] monolayers can be found [here](#)). The wells were then filled with 100 μ l DMEM containing either no serum, 0.5%, 1%, 2%, 5%, or 10% of one of four different serum types (heat inactivated FBS, sterile FBS, BCS or HS). After three days of incubation at 37°C, supernatant was collected, thoroughly mixed and subjected to ELISA assays in order to measure the release of GM-CSF and IL-6 into the medium.

Results

A serum concentration-dependent release of GM-CSF (Figure 1) and IL-6 (Figure 2) can be seen for all serum types with lowest amounts for 0.5% serum and highest amounts for 10% serum. All serum types were able to aid the release of both cargo proteins from the crystals into the medium. A clear conclusion about differences of release capability between the serum types cannot be drawn due to high lot-to-lot variations of sera and their generally undefined and variable composition. The amount of free cargo protein in the medium after release is also dependent on other factors, such as the stability of the cargo protein

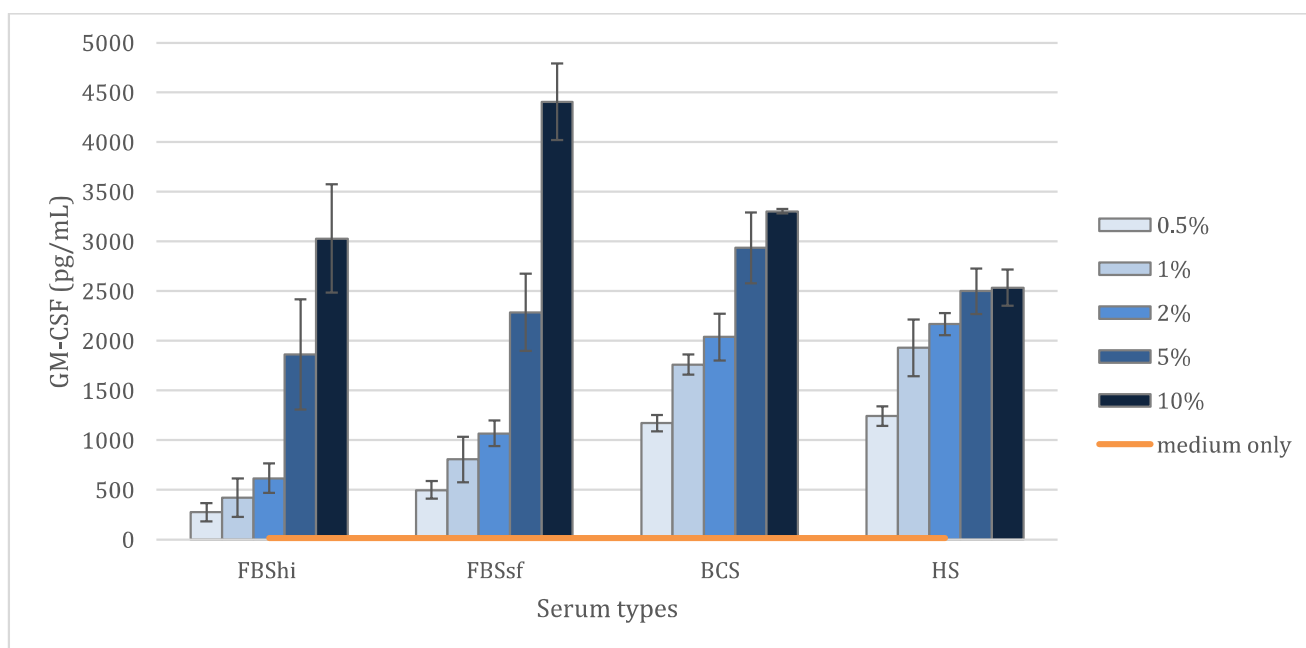


Figure 1: Serum dose-dependent release of GM-CSF from PODS[®] GM-CSF crystals after 3 days. FBSi= FBS heat inactivated, FBSf= FBS sterile filtered.

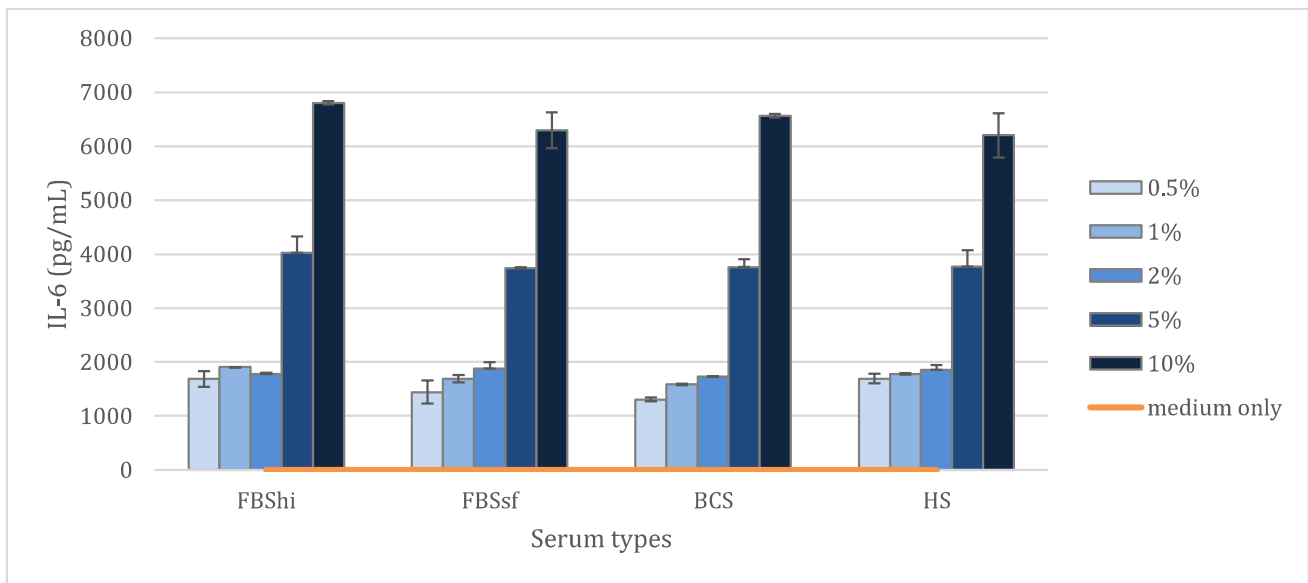


Figure 2: Serum dose-dependent release of IL-6 from PODS® IL-6 crystals after 3 days. FBSHi= FBS heat inactivated, FBSsf= FBS sterile filtered.

Conclusions

- Cargo protein release from PODS® crystals can be modulated by varying serum content in the growth medium.
- Commonly used serum compositions are sufficient to release cargo protein from PODS® crystals in sufficient quantities to achieve useful effects in cell culture

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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
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