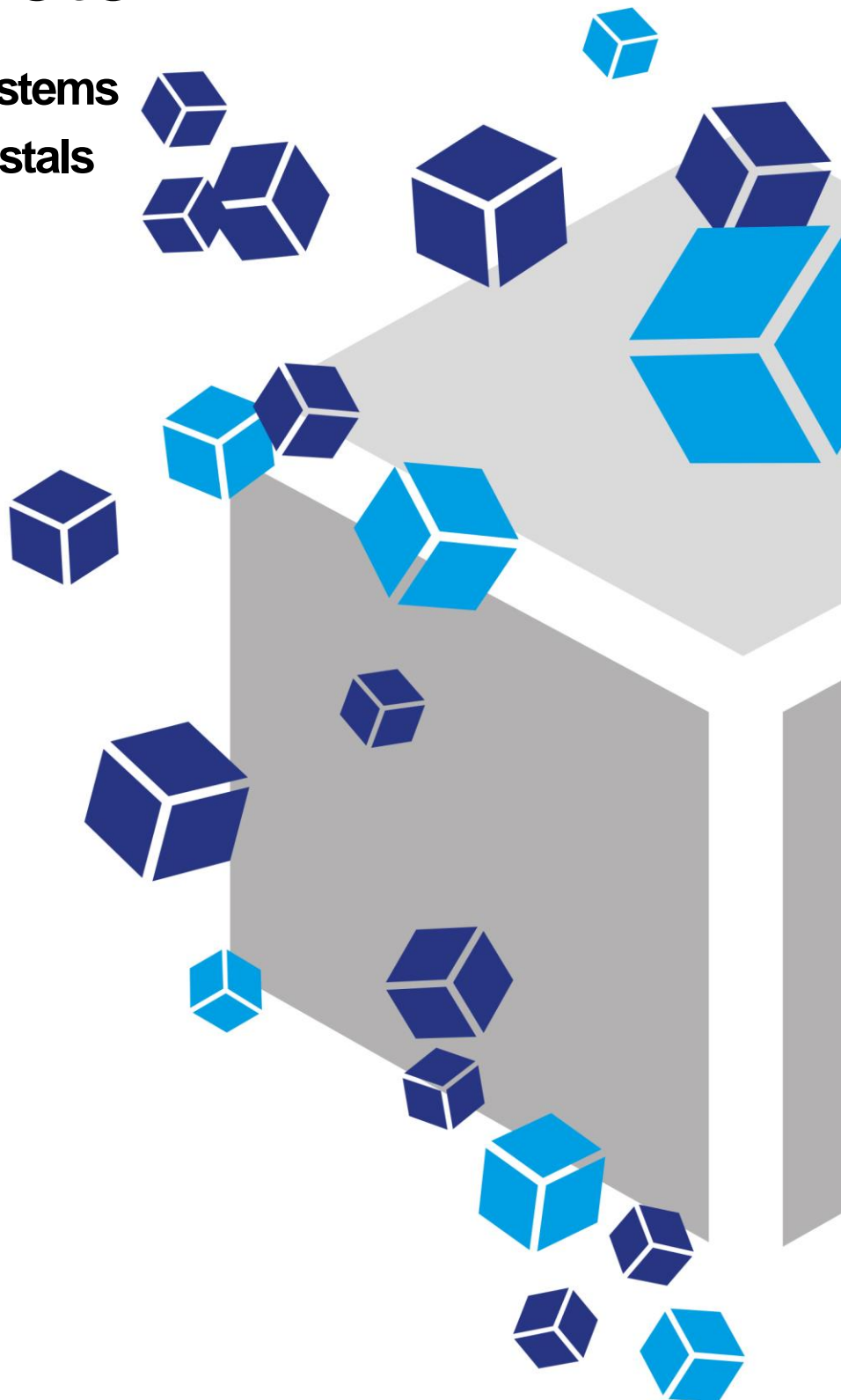


Technical Note

**Configuring culture systems
to maintain PODS[®] crystals
separate from cells**



Configuring culture systems to maintain PODS[®] crystals separate from cells

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

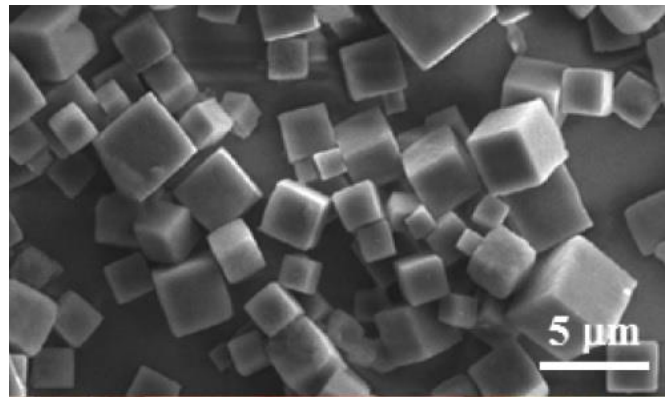
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

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

In some cases, a physical barrier between PODS[®] and cells may be desirable as cells are either (1) affected by contact inhibition; or (2) exhibit phagocytic properties; or (3) require a rapid sequential change of growth factors, as, for example, in differentiation protocols. The utility of cell culture inserts and hydrogels to keep PODS[®] and cells separated during culture in multi-well plates are described and demonstrated in two cell proliferation assays.

Method 1: Placing PODS[®] crystals in tissue culture inserts

PODS[®] GM-CSF were either centrifuged down onto 12-well plates (20 min at 3000 x g) and dried on, to generate uniformly distributed monolayers of PODS[®] crystals (more information on creating even PODS[®] monolayers can be found [here](#)), or were added to cell culture inserts in 200 μ l of growth medium (RPMI + 10% BCS). GM-CSF dependent TF-1 cells (4×10^4 cells/well) were then seeded onto the PODS[®] monolayers or into empty wells (Figure 1A). Subsequently, the PODS[®]-containing inserts were placed into wells that contained only cells (Figure 1B). Plates were then incubated for 5 days at 37°C with 5% CO₂. Subsequently, TF-1 cell numbers for each well were measured by performing a colorimetric cell counting assay ([Orangu[™]](#), Cell Guidance Systems). 2 ng/ml conventional GM-CSF was used as a technical control.

Results

TF-1 cells show a dose-dependent increase in proliferation when grown in the presence of PODS[®] GM-CSF crystals. Cells cultured in the direct vicinity of 2×10^6 PODS[®] GM-CSF crystals increased 5-fold compared to cells grown without growth factor, similar to cells grown with 2 ng/ml conventional recombinant GM-CSF. Cells cultured in the presence of inserts containing 2×10^6 PODS[®] GM-CSF crystals increased 3-fold, showing an efficiency of at least 60% compared to cells cultured next to PODS[®]. The difference between PODS[®] GM-CSF crystals in inserts and PODS[®] in the same well as the cells can be attributed to the physical distance between cells and PODS[®] in the insert set-up.

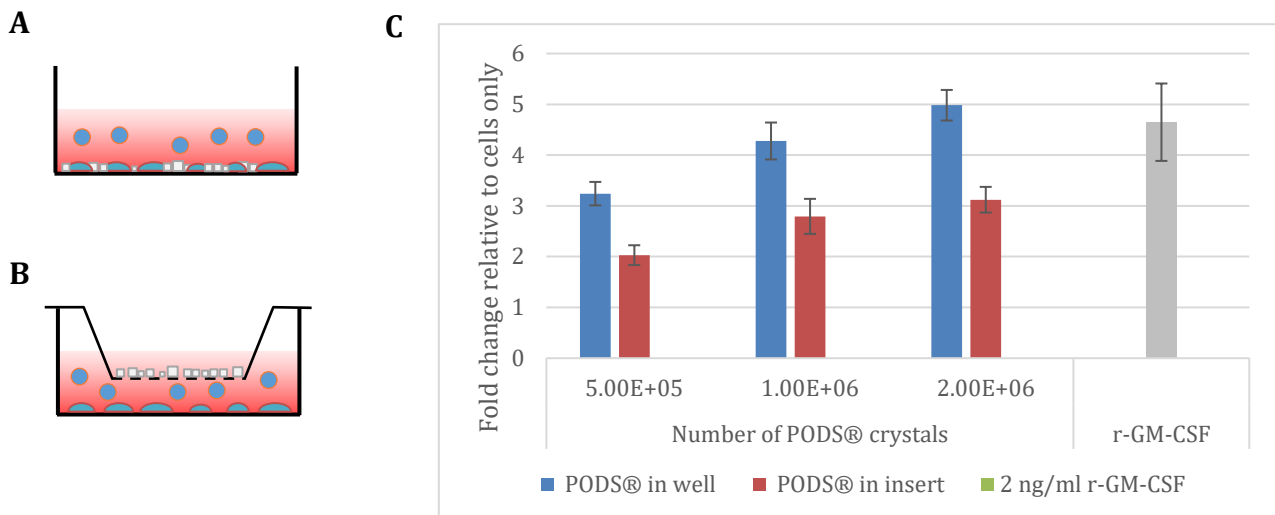


Figure 1: Dose-dependent proliferation of GM-CSF dependent TF-1 cells in the presence of PODS[®] GM-CSF crystals. TF-1 cells were incubated in 12-well plates either with (A) the PODS[®] GM-CSF crystals directly in the well; or (B) together with the PODS[®] GM-CSF crystals in inserts. (C) After 5 days, proliferation was measured by performing a colorimetric assay. Conventional human recombinant GM-CSF served as a technical control for TF-1 cell proliferation.

Method 2: PODS® separated from cells using collagen Type I-A hydrogel

PODS® GM-CSF were centrifuged onto 96-well plates (20 min at 3000 x g) and dried on, to generate uniformly distributed monolayers of PODS® crystals. Half of the wells were covered with 50 µl of collagen Type I-A gel (Cellmatrix® Collagen Type-I-A, FUJIFILM) (Figure 2B). GM-CSF-dependent TF-1 cells (2×10^4 cells/well) were then seeded in growth medium onto the gel-covered and naked PODS® monolayers. Plates were then incubated for 5 days at 37°C with 5% CO₂. Subsequently, TF-1 cell numbers for each well were measured by performing a colorimetric cell counting assay (Orangu™, Cell Guidance Systems).

Results

TF-1 cells grown on collagen I-A gels covering a PODS® GM-CSF monolayer show a 10-fold increase in proliferation compared to cells grown on collagen without any growth factor. The difference in fold-change between PODS® GM-CSF crystals under collagen I gels and naked PODS® can be attributed to endogenous proteases contained in collagen aiding in the breakdown of PODS® crystals and thus the release of cargo.

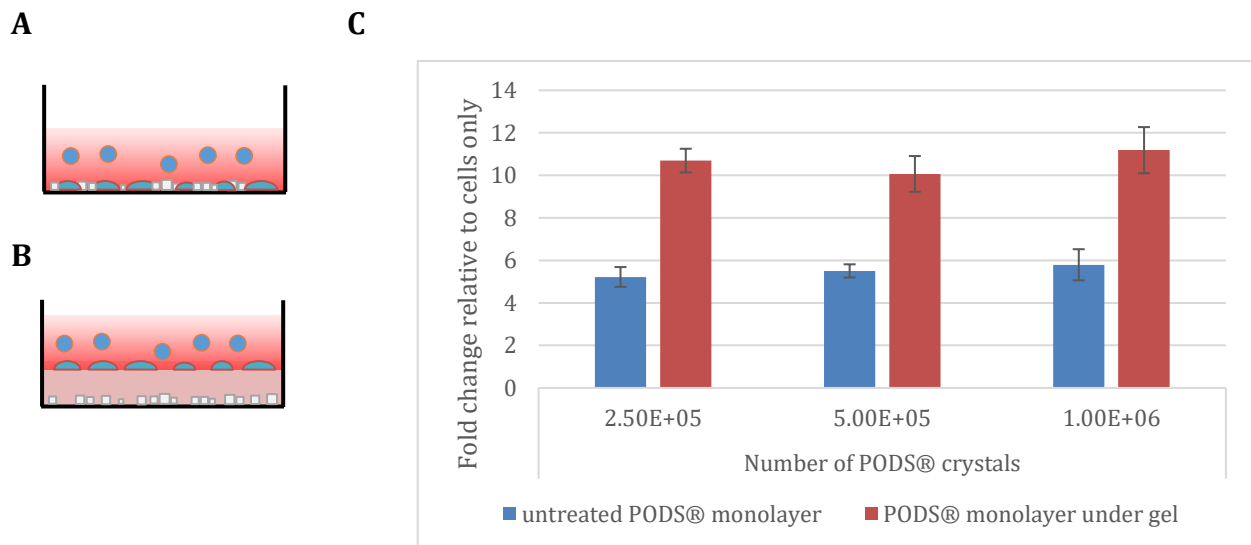


Figure 2: Proliferation of GM-CSF-dependent TF-1 cells in the presence of PODS® GM-CSF crystals. TF-1 cells were incubated in 96-well plates with either (A) the PODS® GM-CSF crystals in the well next to the cells; or (B) with PODS® GM-CSF under a layer of collagen I-A gel. (C) After 5 days, proliferation was measured performing a colorimetric assay. Human recombinant GM-CSF served as a positive control for TF-1 cell proliferation. Both conditions are normalised to their respective cells-only control, either grown on untreated tissue culture plastic or collagen I-A gel.

Conclusions

- Simple physical separation of cells and PODS® crystals is possible with standard cell culture inserts or using hydrogel
- Cells can be used in downstream experiments without carryover of PODS®
- Inserts are a practical way for rapid removal of PODS® crystals from cell cultures (e.g. in differentiation protocols) with minimal loss of efficiency compared to a direct use of PODS®
- PODS® crystals in combination with animal-derived hydrogels such as collagen can increase cargo release from PODS® further

For more information and a full list of our current PODS® growth factors, please visit our website www.cellgs.com.



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

Growth Factors

- Recombinant
- Sustained Release

Exosomes

- Purification
- Detection
- NTA Service

Small Molecules

Cell Counting Reagent

Matrix Proteins

Cell Culture Media

- Photostable
- Custom Manufacturing Service

Cytogenetics Analysis



General info@cellgs.com
Technical Enquiries tech@cellgs.com
Quotes quotes@cellgs.com
Orders order@cellgs.com

www.cellgs.com

EUROPE

Cell Guidance Systems Ltd
Maia Building
Babraham Bioscience Campus
Cambridge
CB22 3AT
United Kingdom
T +44 (0) 1223 967316
F +44 (0) 1223 750186

USA

Cell Guidance Systems LLC
Helix Center
1100 Corporate Square Drive
St. Louis
MO 63132
USA
T 760 450 4304
F 314 485 5424