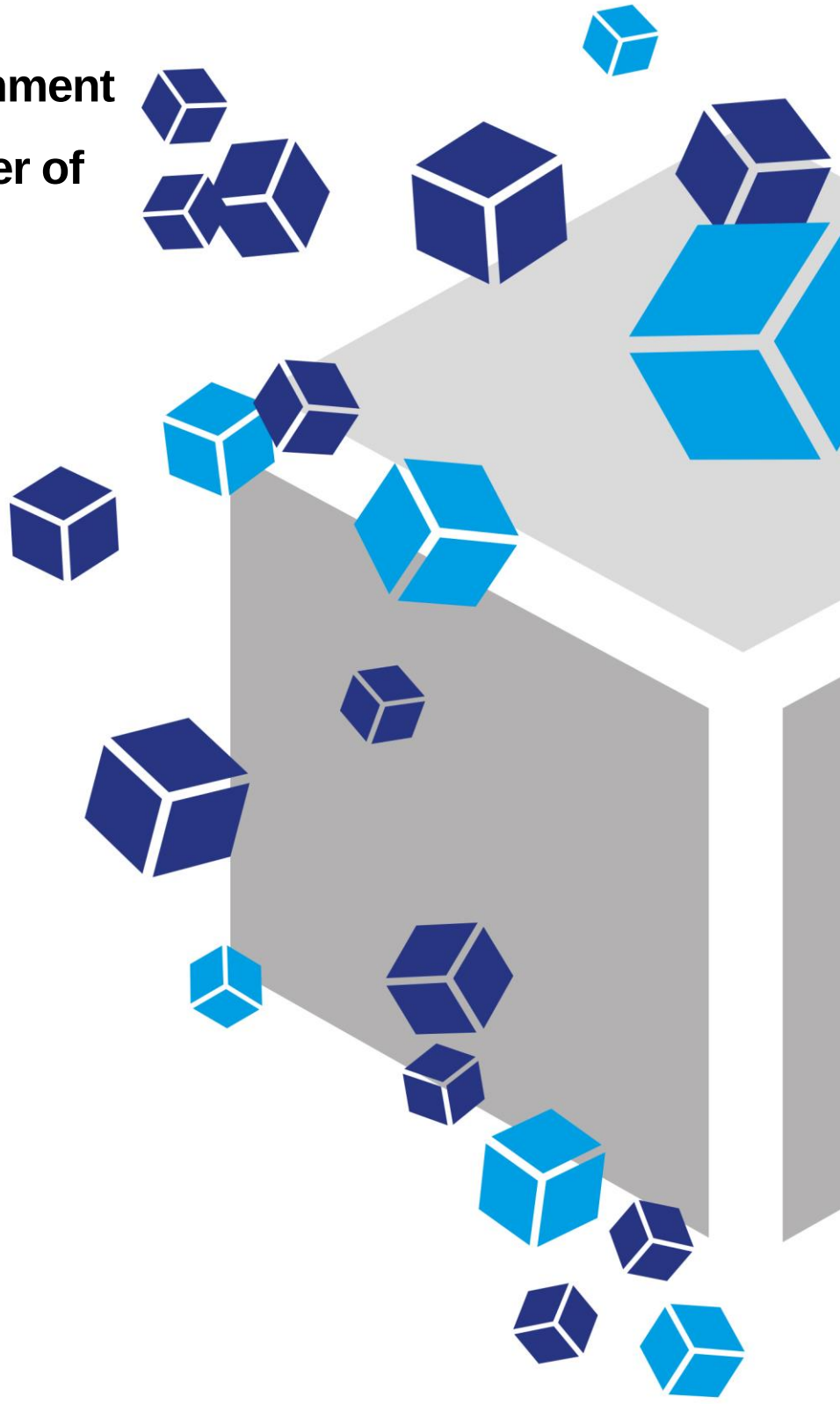
Technical Note

Enzymatic cell detachment with minimal carryover of PODS® crystals



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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 suffer from lot-to-lot product variation. This fragility and variability hampers research and significantly limits the therapeutic potential of proteins.

Protein Micro-depots

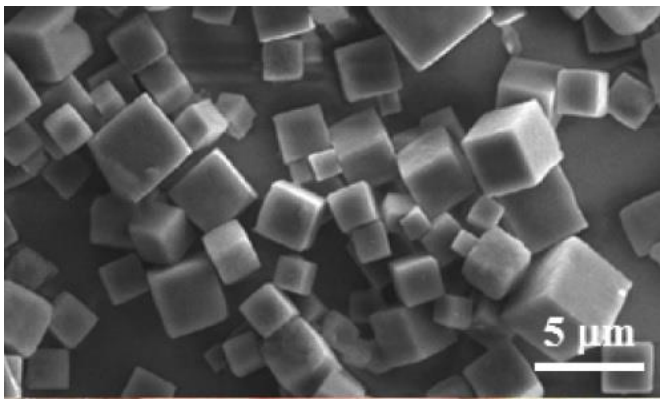
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

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

It is sometimes necessary to remove growth factors from a culture system. Therefore, it is important to separate cells from PODS® which provide sustained growth factors release. This can be achieved in one of two ways. Firstly, by placing the PODS® crystals in a device that can be removed, such as a transwell insert. Secondly, by removing the cells during passaging. Enzyme-based dissociation buffers are commonly used for detaching a wide range of adherent mammalian cells. Dissociated cells are then used either for regular propagation (passaging) or subsequent downstream applications, where cells need to be detached from cell culture surface material for analysis. Therefore, detaching cells without dislodging adherent PODS® crystals to prevent carry-over is an important step in the experimental workflow. Following treatment with TrypLE, a modified trypsin detachment buffer, the adherence of PODS® crystals to plastics was assessed by first attaching PODS® eGFP to cell culture vessels and then employing a standard cell dissociation protocol.

Methods

The maximum recommended number of PODS® eGFP crystals (3x 10^6) were centrifuged onto a 24-well plate (20 min at 3000 x g) and allowed to dry, generating uniformly distributed monolayers of PODS® crystals (more information on creating uniform PODS® layers can be found here). Full growth medium (DMEM + 10% serum) with or without NIH-3T3 cells (3x 10^3) was added to the wells by carefully pipetting against the side of the well, and incubated for 5 days. Subsequently, media was removed and replaced by either 600 µL PBS or 600 µL TrypLE (#12604021, Gibco) and incubated for 3 min at 37°C with 5% CO_2. Supernatant with dislodged PODS® crystals from each condition was collected and transferred into a 96-well plate for downstream analysis. As a reference, 3x 10^4 PODS® eGFP crystals (i.e. equivalent to 1% of the PODS® starting material) was plated into empty wells of the same 96-well plate and the eGFP signal was measured using a fluorescence plate reader. The number of PODS® crystals lost during dissociation was determined as a percentage of the reference.

Results

Loss of PODS® crystals during enzymatic cell detachment

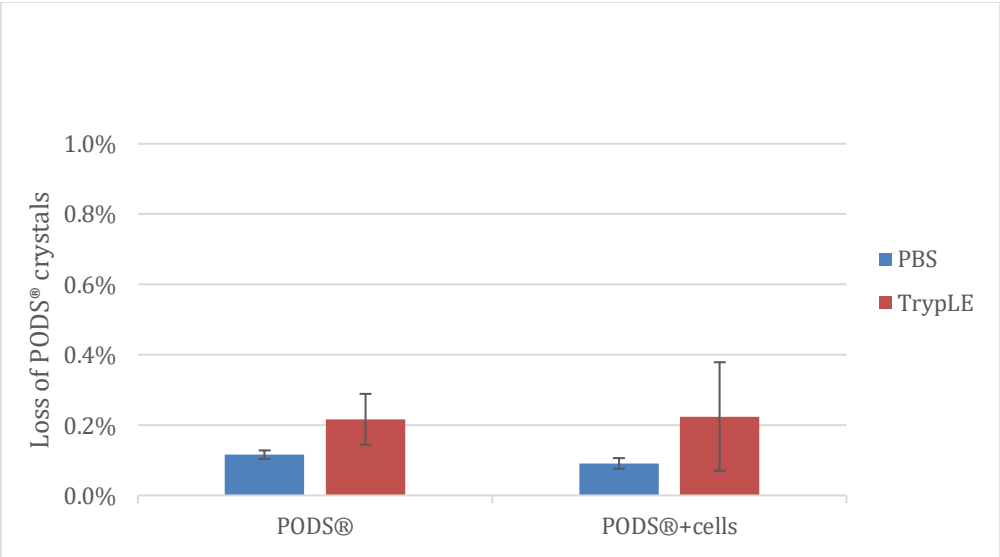


Figure: Assessment of carryover of PODS® crystals during detachment. A trypsin-based detachment procedure was carried out on a PODS®-coated tissue culture dish in the absence (left) and presence (right) of murine fibroblast (NIH-3T3) cells. In either experiment, TrypLE had a negligible impact on removing PODS® from tissue culture surfaces, comparable to a simple wash with PBS. The data indicates that there is no quenching of the eGFP signal by the cells.

Conclusions

- Ease of use: PODS® crystals are compatible with standard enzymatic cell dissociation buffers and procedures
- There is minimal carryover of PODS® crystals during cell passaging and into downstream experiments

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

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