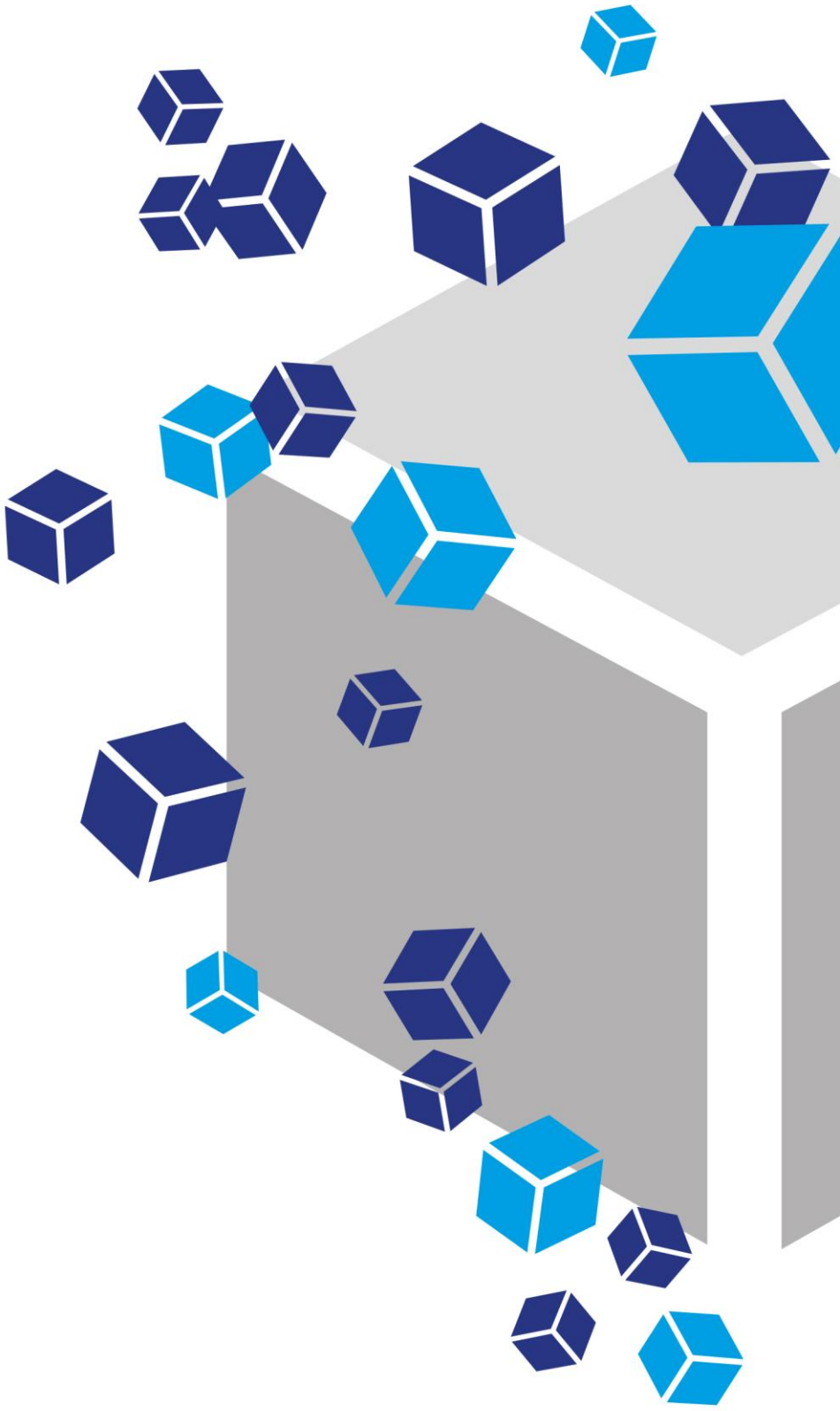
Application Note

Modulating protein release rates from PODS® crystals



Pre-incubating PODS® crystals to modulate release of GM-CSF

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

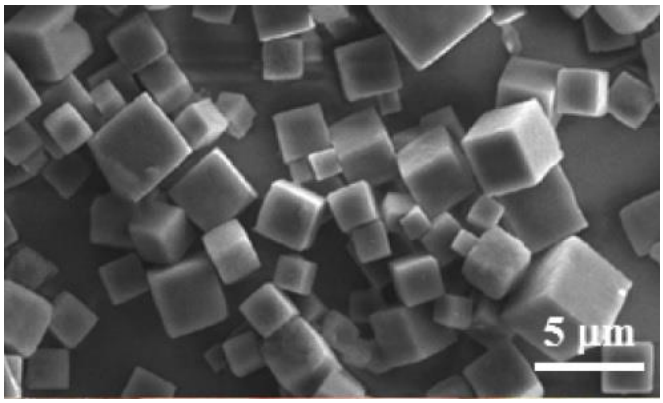
Development of a technology that can continuously replenish active protein from a local, microscopic store has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control over the growth of cells.

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

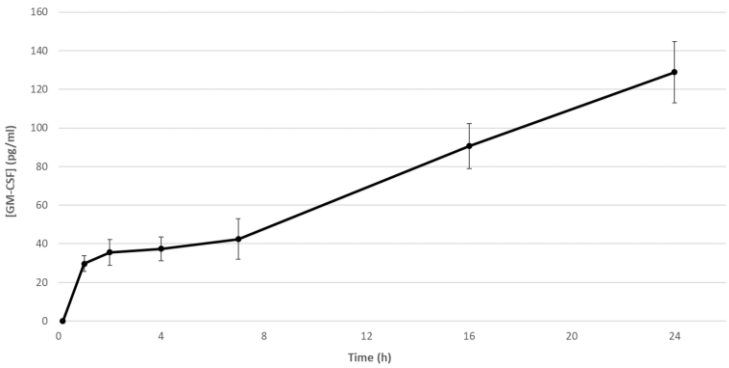
Methods

Pre-incubation: PODS® GM-CSF crystals and PODS® Empty crystals (5x 10^5) were spotted into wells of a 96-well plate and dried on. Subsequently, RPMI + 10% FBS was added to each well and incubated at 37°C.

Culture method: PODS® GM-CSF crystals and PODS® Empty crystals were spotted into wells as described above. TF-1 cells, which are dependent on GM-CSF, were then seeded and cultured for 5 days. **NOTE:** a single application of PODS® crystals was used during the culture period without any media change.

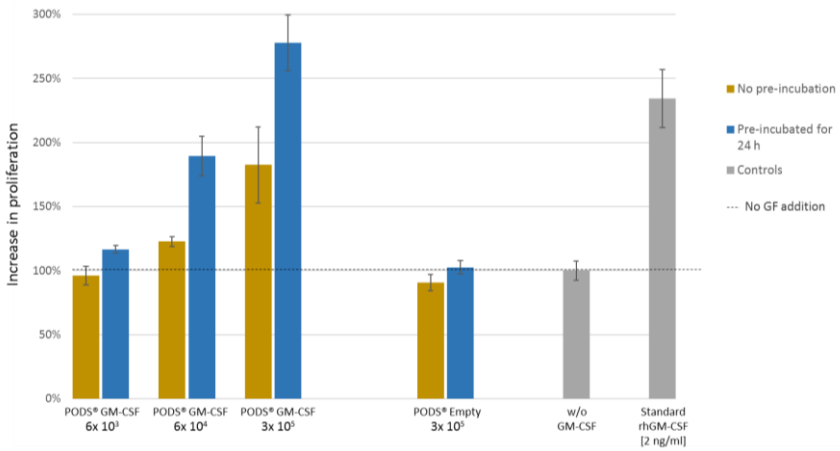
Cell counting: TF-1 cell number was assessed using the cell counting solution Orangu™ (Cat OR01) according to the guidelines. Briefly, 10 µl of Orangu™ solution per 100 µl of cell culture medium was added to each well. The plate was then incubated at 37°C for 2 hours, and subsequently centrifuged at 3000 *xg* for 20 minutes to prevent carry-over of PODS® crystals. The supernatant was transferred into wells of a fresh plate and the absorbance measured at 450 nm using a microplate reader.

Results



Release of GM-CSF over 24 hours from PODS® GM-CSF crystals, quantified by ELISA. PODS® GM-CSF crystals (5x 10^5) were spotted onto 96-well plates and dried on. Subsequently, RPMI + 10% FBS was added to each well and incubated at 37°C. Medium was removed at indicated time points. GM-CSF was quantified by ELISA. Error bars represent 3 technical repeats.

Proliferation of TF-1 cells in the presence of PODS® GM-CSF with or without pre-incubation. PODS® GM-CSF crystals or PODS® Empty crystals were spotted onto a 96-well plate, after which RPMI + 10% FBS was added and incubated for 24 h. Subsequently, 2x 10^3 TF-1 cells in RPMI + 10% FBS were directly seeded on top and incubated for a further 5 days (blue bars). Cell number was assessed using a colorimetric assay, and proliferation was plotted relative to unsupplemented TF-1 cells. Error bars represent 8 technical repeats.



Conclusions

- Serum-containing cell culture medium can activate the release of cargo protein from PODS® crystals.
- Pre-incubating PODS® crystals provides a starting amount of cargo protein in culture medium, beneficial if an initial supply of protein is critical.
- A single application of PODS® crystals is effective, significantly reducing both hands-on time and cost of materials.

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*

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- Sustained Release

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- NTA Service

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Gene Knock-Up System

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