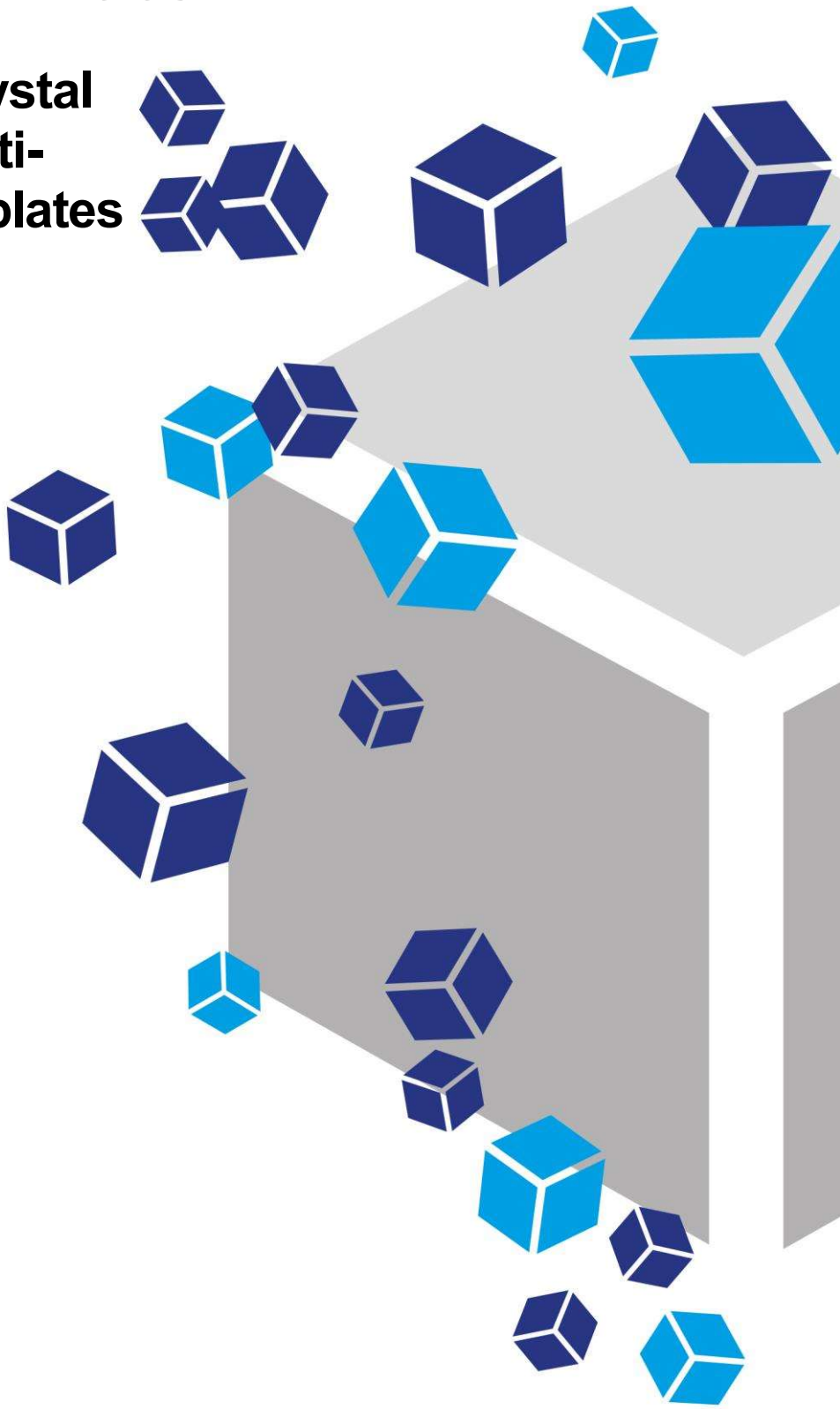
CELL guidance systems

Application Note

Creating PODS® crystal monolayers on multi-well tissue culture plates



Creating PODS® crystal monolayers on multi-well tissue culture plates

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

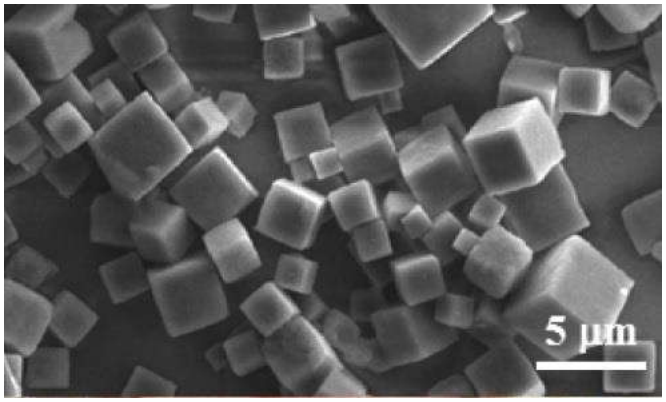
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control over the growth of cells.

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

Adhering PODS® crystals to tissue culture (TC) multi-well plates or inserts

1. Dilute PODS® crystals into PBS and pipette the suspension into wells of a TC multi-well plate or TC inserts (see table below for recommended PODS® crystal amounts and PBS volumes).
2. Spin TC multi-well plate or inserts in wells in a centrifuge with plate rotor for 20 minutes at 3000 x *g*.
3. Remove supernatant and allow PODS® crystals to dry onto wells/inserts, either by leaving the plate at room temperature for 1 h with the lid removed or overnight in the fridge with the lid closed.
4. Add cell suspension or media to wells by gently pipetting against the side of the well.

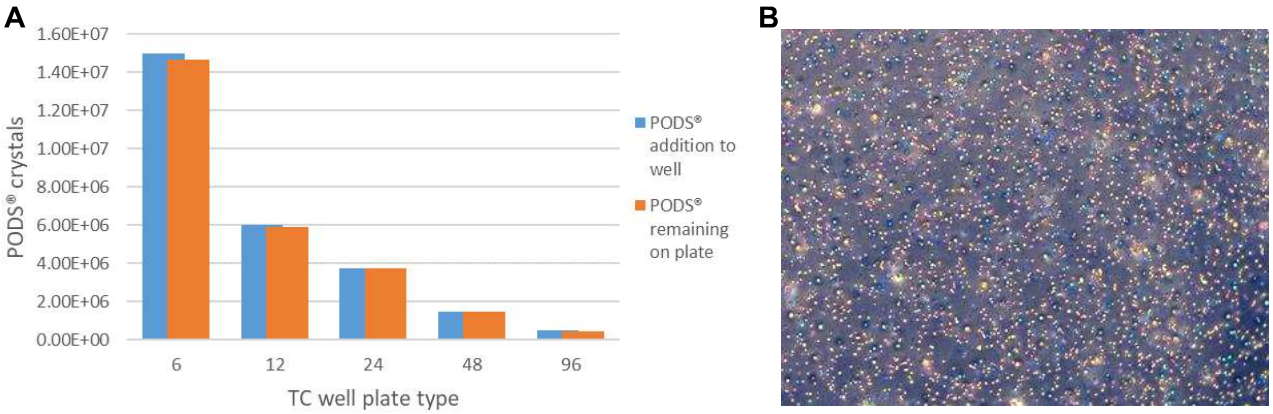
Results

Recommended maximum PODS® crystal amounts and PBS volumes to create PODS® monolayers by centrifugation

Plate type	Area (cm^2)	Recommended PBS [µl]	Maximum recommended PODS®*	Loss of PODS® after supernatant removal (%)	Loss of PODS® after a full media change (%)
6	9.50	1000	$1.5x10^7$	2.0	4.0
12	3.80	600	$6.0x10^6$	1.5	3.0
24	1.90	300	$3.0x10^6$	0.5	1.5
48	0.95	150	$1.5x10^6$	1.0	9.0**
96	0.32	50	$5.0x10^5$	3.0	5.0

* Higher densities of PODS® crystals can be achieved but will result in higher subsequent losses.
** Higher loss of PODS® crystals is due to the geometry of a 48-well plate that prevents pipetting to the side of the well.

PODS® crystals adhered to TC plastic surfaces



Maximum recommended amounts of PODS® crystals were spun onto various TC plates.
(A) Retention of PODS® crystals on TC plastic surfaces were measured after aspiration of supernatant.
(B) Brightfield micrograph of a monolayer of dispersed PODS® crystals at the maximum recommended density.

Conclusions

- PODS® crystals adhered to TC surfaces as a monolayer will release growth factor evenly across the well.
- Uniformly distributed monolayers of PODS® crystals on TC plastic can be easily achieved using a centrifuge with plate rotor.
- Crystal densities of up to $1.4x10^6$ PODS®/cm^2 will readily adhere to TC surfaces as a well-spaced out single layer.
- Medium changes can be performed by simply aspirating conditioned medium as usual without significant loss of PODS® crystals.

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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