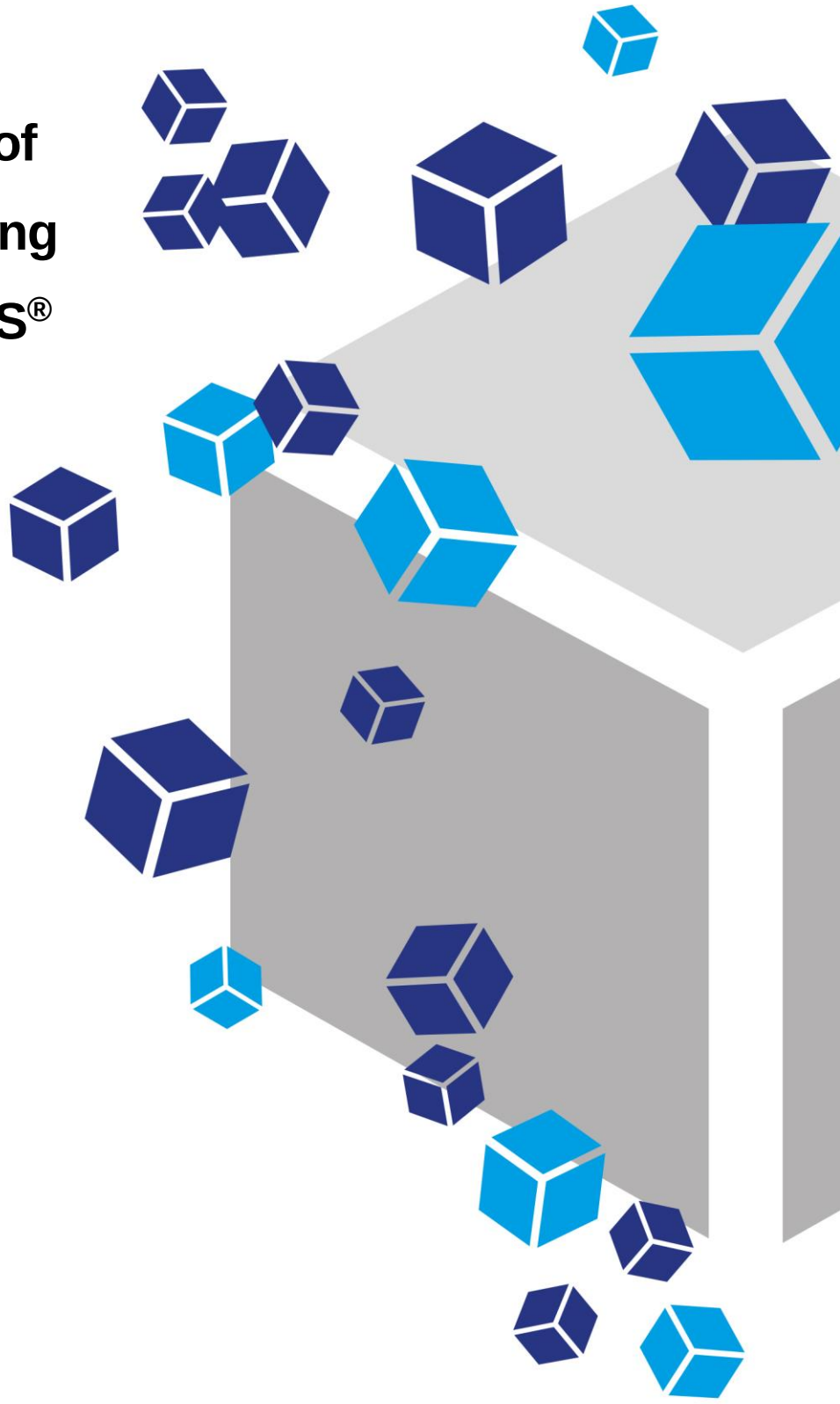
CELL guidance systems

Technical Note

Differential effects of tissue culture coating substrates on PODS® cargo release



Differential effects of tissue culture coating substrates on PODS® cargo release

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

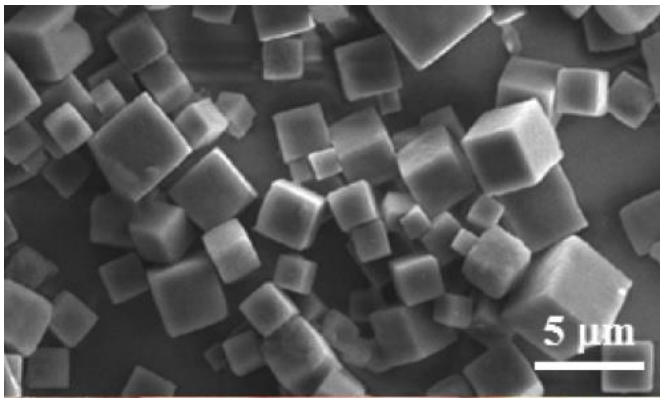
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

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

Extracellular Matrix (ECM) proteins used for coating tissue culture (TC) plastic surfaces have shown to be important for regulating cellular behaviour, cell attachment, proliferation, shape, migration and differentiation of various cell types. PODS® crystals provide sustained levels of growth factor from a single dose to cells, however, positioning PODS® crystals above or underneath various 2D TC coating materials can affect release rates of PODS® cargo growth factors.

To measure the effects of different TC coating materials on PODS® growth factor release, PODS® were localized above or below various common coating substrates (Vitronectin, Laminin 521 and Matrigel®) and cultured for 7 days in serum-containing medium. ELISA was used to assess the amount of cargo protein released from PODS® crystals. The data presented here demonstrates more rapid PODS® growth factor release when PODS® crystals are localized on top of 2D TC coating substrates.

Methods

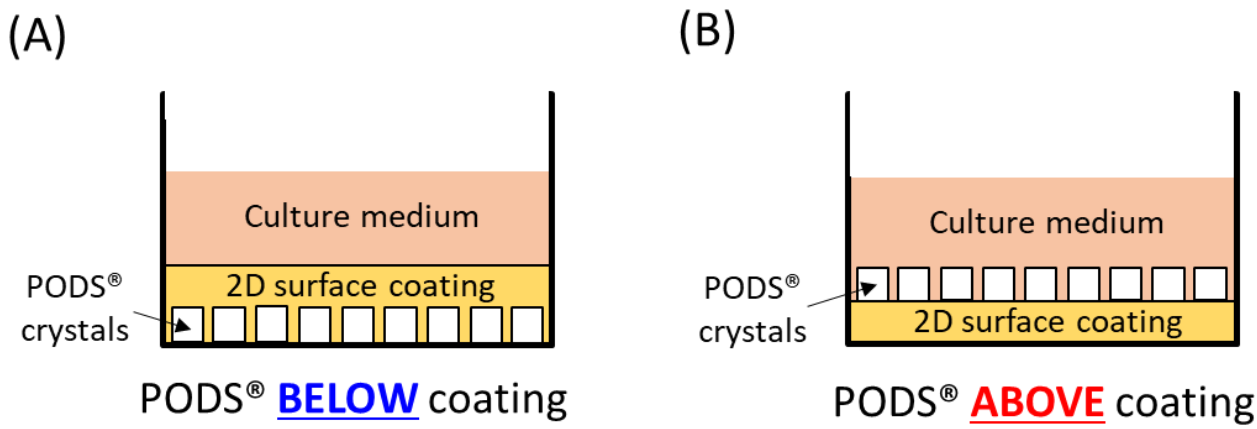


Figure 1: Localization of PODS® crystal layers (A) below or (B) above various 2D TC plastic coating substrates. (A) PODS® IL-06 crystals (1 x 10^6/well) were dried onto 96-well plates. Then the crystal layer was coated on top with individual coating substrates, either Vitronectin; Laminin 521; or Matrigel®. Culture medium (DMEM + 10% Fetal Bovine Serum [FBS]) were added to each well and incubated at 37°C for 7 days. (B) Alternatively, TC plastic was initially coated with either Vitronectin; Laminin 521; or Matrigel®. Then, PODS® IL-06 crystals (1 x 10^6/well) were seeded on top of the substrate coating layer. Medium was added to each well and incubated at 37°C for 7 days.

Adhering PODS® crystals below coating substrates (Figure 1A):
PODS® crystals were dried onto the surface of tissue culture plates using the method described in another Technical Note. Briefly, a dense layer of PODS® IL-06 (catalogue number: PPH10) containing 1 × 10^6 PODS®/well was attached to each well by first pipetting 50 µl of PODS® solution into wells of a 96-well plate, then centrifuging for 20 minutes at 3000 × g. The supernatant was then removed and the crystals left to dry on to the plate. Next, TC surface coating substrates: either Human Recombinant Vitronectin (5 µg/ml; Thermofisher Scientific; A31804); Recombinant Laminin 521 (5 µg/ml; Stemcell Technologies; # 77003) or Matrigel® Growth Factor Reduced (145 µg/ml; Corning; 356230) were diluted according to manufacturers' recommendations and 50 µl/well of solution was carefully added to each well. The plates were incubated for 1 hour at room temperature. The solution was then removed and then 100 µl culture medium (DMEM containing 10% FBS) was added per well. PODS® crystals were incubated at 37°C for 7 days.

Adhering PODS® crystals above coating substrates (Figure 1B):
Either, Matrigel® Growth Factor Reduced (145 µg/ml; Corning; 356230); or Recombinant Laminin 521 (5 µg/ml; Stemcell Technologies; # 77003); or Human Recombinant Vitronectin (5 µg/ml; Thermofisher Scientific; A31804) were diluted according to manufacturers' recommendations and 50 µl/well of solution was added into wells of a 96-well plate. Plates were incubated for 1 hour at room temperature. Next, the coating solution was removed, diluted PODS® IL-06 (1×10^6 PODS® /well; catalogue number: PPH10) were immediately added into each well and then centrifuged for 20 minutes at 3000 × g. Finally, supernatant was removed and 100 µl culture medium was carefully added to each well. PODS® crystals were incubated at 37°C for 7 days.

Analysis:
Following collection of samples for analysis, ELISA assays (SEKB10395, SinoBiological) were performed to measure the levels of growth factor released from the PODS® crystals into the medium.

Results

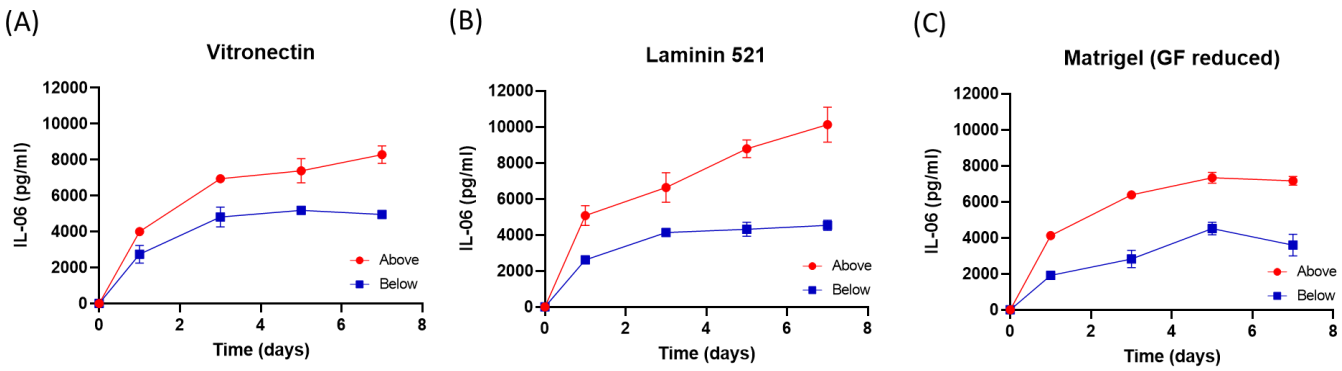


Figure 2: Release of IL-06 from PODS® IL-06 crystals localized above or below either (A) Vitronectin- (B) Laminin 521- and (C) Matrigel- 2D coating substrates, incubated over 7 days with serum-containing medium. Medium was removed at indicated time points and IL-06 concentrations (pg/ml) were quantified by ELISA. Error bars represent standard error mean calculated from 3 technical repeats.

Conclusions

- PODS® growth factors are highly compatible adhering well to both uncoated TC plastic and substrates such as Vitronectin, Laminin 521 or Matrigel®.
- Greater release from PODS® growth factors could be detected when PODS® crystals are localized on top of 2D TC coating substrates.
- Coating type does not influence release rates of cargo protein from PODS® crystals when cultured on different coating substrates.

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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