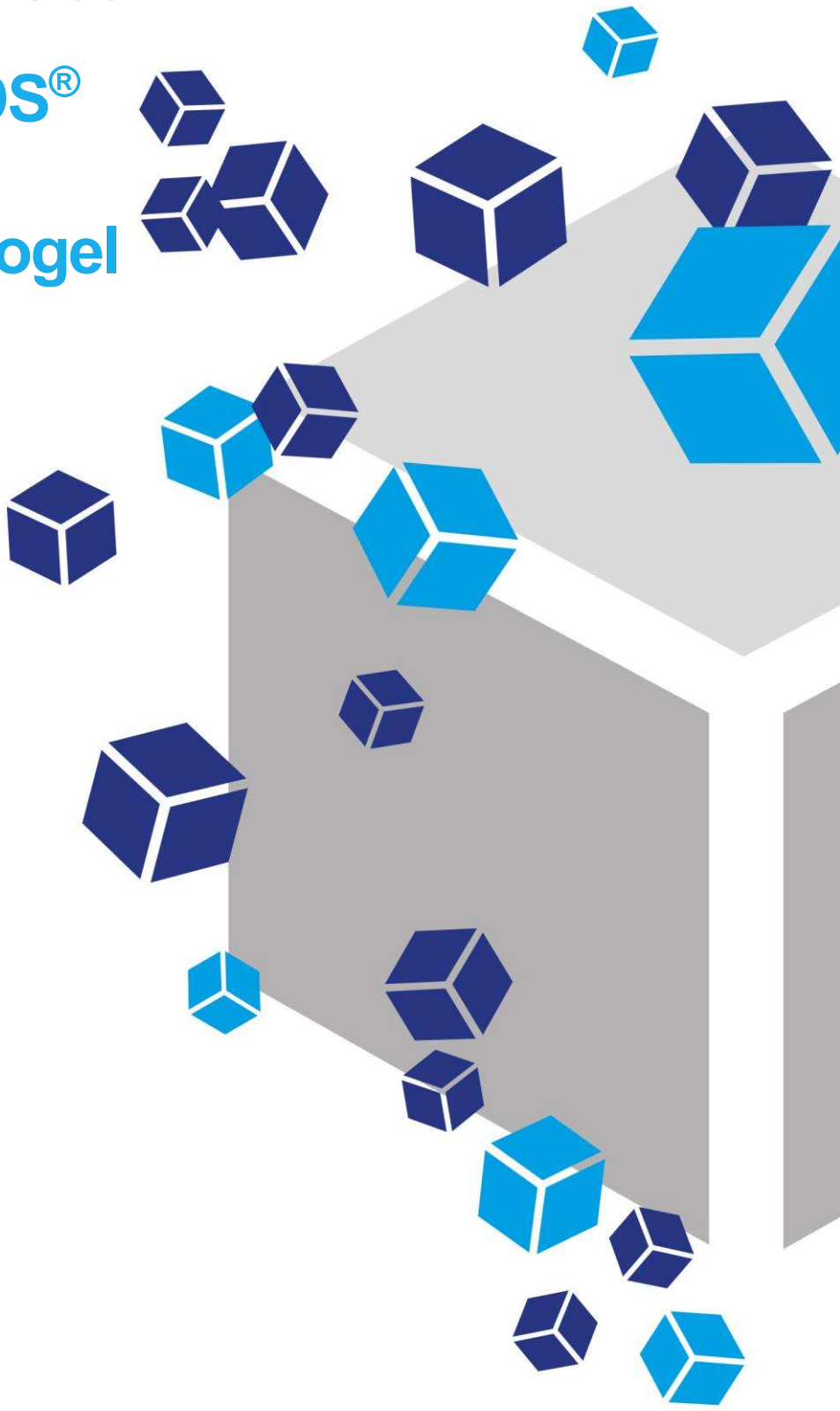
CELL guidance systems

Technical Note

Integrating PODS® proteins into GrowDex® hydrogel



Integrating PODS® proteins into GrowDex® hydrogel (Issued July 2021)

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

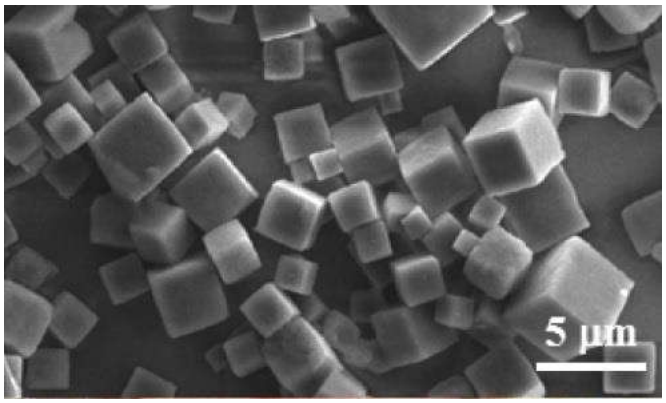
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control over the growth of cells.

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

Overview

Traditionally, cells are cultured as a monolayer on the surface of tissue culture-treated flasks and well plates. In the past decade it has become increasingly clear that cells cultured in 3D, e.g. as spheroids, organoids or on scaffolds, have an increased physiological relevance compared to 2D cell culture, specifically in drug discovery research. Often 3D culture is facilitated by the application of a hydrogel into which cells can be embedded, either as a dispersed population or as spheroids or organoids. While animal-derived hydrogels such as collagen or Matrigel® are still commonly used, these suffer from several drawbacks including batch-to-batch variability, and that they are chemically ill-defined. A growing number of non-animal alternatives to Matrigel® have been developed. This includes the GrowDex® range of gels from UPM Biomedicals. GrowDex® are animal-free hydrogels derived from birch wood. As such they are inherently biocompatible and biodegradeable, with excellent batch-to-batch variability, animal and disease-free, and thus improve on the reliability and consistency of results over aforementioned animal-derived matrices.

Functionalizing GrowDex® by embedding PODS® proteins into the gel matrix provides localised depots of growth factor (GF), mimicking effects seen in tissues by surrounding the cells within the hydrogel. PODS® embedded in hydrogels release bioactive GF for several weeks (see Technical Note), reducing the need for medium changes and hence handling frequency, which is particularly useful for complex cultures that require minimum disturbance. This study demonstrates that PODS® can be easily combined with GrowDex® gels, without changing gel characteristics or GF release from the PODS® crystals.

Methods

Adhering PODS® crystals to tissue culture (TC) multi-well plates or inserts: PODS® crystals were dried onto the surface of tissue culture plates using the method described in a previous Technical Note. Briefly, dense layers of PODS® IL-06 (catalogue number PPH10) containing 1×10^5, 5×10^5, or 1×10^6 PODS®/well (3.125×10^5, 15.625×10^6, or 3.125×10^6 PODS®/cm^2) were created by first pipetting 100 µl of PODS® solution into each well of a 96-well plate, then centrifuging for 20 minutes at 3000 × g. The supernatant was then removed and the crystals left to dry before adding media and/or gel.

Gel casting onto PODS® crystal layer: Gels (GrowDex® or GrowDex®-T; Collagen I, Nitta Gelatin Inc.) were prepared according to the manufacturers' instructions, and cast into the wells of the TC multi-well plate above the dried PODS® crystal monolayer. Briefly, GrowDex® was removed from the fridge and pre-warmed to room temperature. It was then diluted 4:1 in culture medium (DMEM) and gently mixed with a positive displacement pipette. Finally, 50 µl of gel was dispensed into each well above the PODS® layer. The plate was incubated at 37 °C for 30 minutes, then 100 µl of media was added on top of each gel.

Gel casting with embedded PODS®: Appropriate PODS® volumes were aliquoted from stock and placed into a microcentrifuge tube. These were centrifuged at 3000 x *g* for five minutes to pellet the PODS®, then supernatant was removed. Media was added in a volume equal to 1/5 of the final gel volume to the PODS® and vortexed to resuspend. The remaining volume (4/5 of the final volume) of GrowDex® was added to this suspension and the PODS® were mixed into the gel by pipetting up and down with a positive displacement pipette. 50 µl of PODS®-GrowDex® mix was dispensed into each well of a 96-well plate. The plate was incubated at 37 °C for 30 minutes, then 100 µl of media was added on top of each gel.

ELISA analysis: Following collection of samples for analysis, ELISA assays (SEKB10395, SinoBiological) were performed according to the manufacturer's instructions to measure the levels of growth factor in the supernatant.

PrestoBlue measurement: at the desired time point, PrestoBlue reagent was added to each well with cells to reach a 1:10 dilution with media (i.e. 10 µl added to 100 µl of media). After incubation for 1 hour, the media was removed and fluorescence was measured using a plate reader (Ex/Em 560/590 nm).

Results

PODS® were combined with GrowDex® gels and evaluated for short-term and long-term integrity as well as for growth factor release under various conditions. PODS® remained well-dispersed throughout the gels in all three dimensions without any sedimentation during 3 days of incubation (Fig. 1). PODS® distribution was unchanged during the 28-day observation period (data not shown).

The release characteristics of cargo protein from PODS® were compared between different experimental conditions, either PODS® without gel; or under the gel; or within gel. Release of GF from PODS® crystals could be modulated by varying the placement of PODS® crystals (Fig. 2).

While release from PODS® could be detected using ELISA in serum-containing conditions (Fig. 2), there was no growth factor release from PODS® observed in serum-free conditions and in the absence of cells, i.e. in the absence of a source of proteases (Fig. 3). This demonstrates that GrowDex® is protease free.

Biological effects of PODS®-functionalized GrowDex-T® were evaluated using a PrestoBlue assay. TF-1 cells, which require the presence of a growth factor such as GM-CSF (CSF-2) to maintain survival were embedded into GrowDex-T® gels either with PODS® CSF-2, with conventional CSF-2, or without any growth factor supplement. PODS® maintained TF-1 cell survival over 4 days whereas in the other two conditions a decrease in cellular activity was observed.

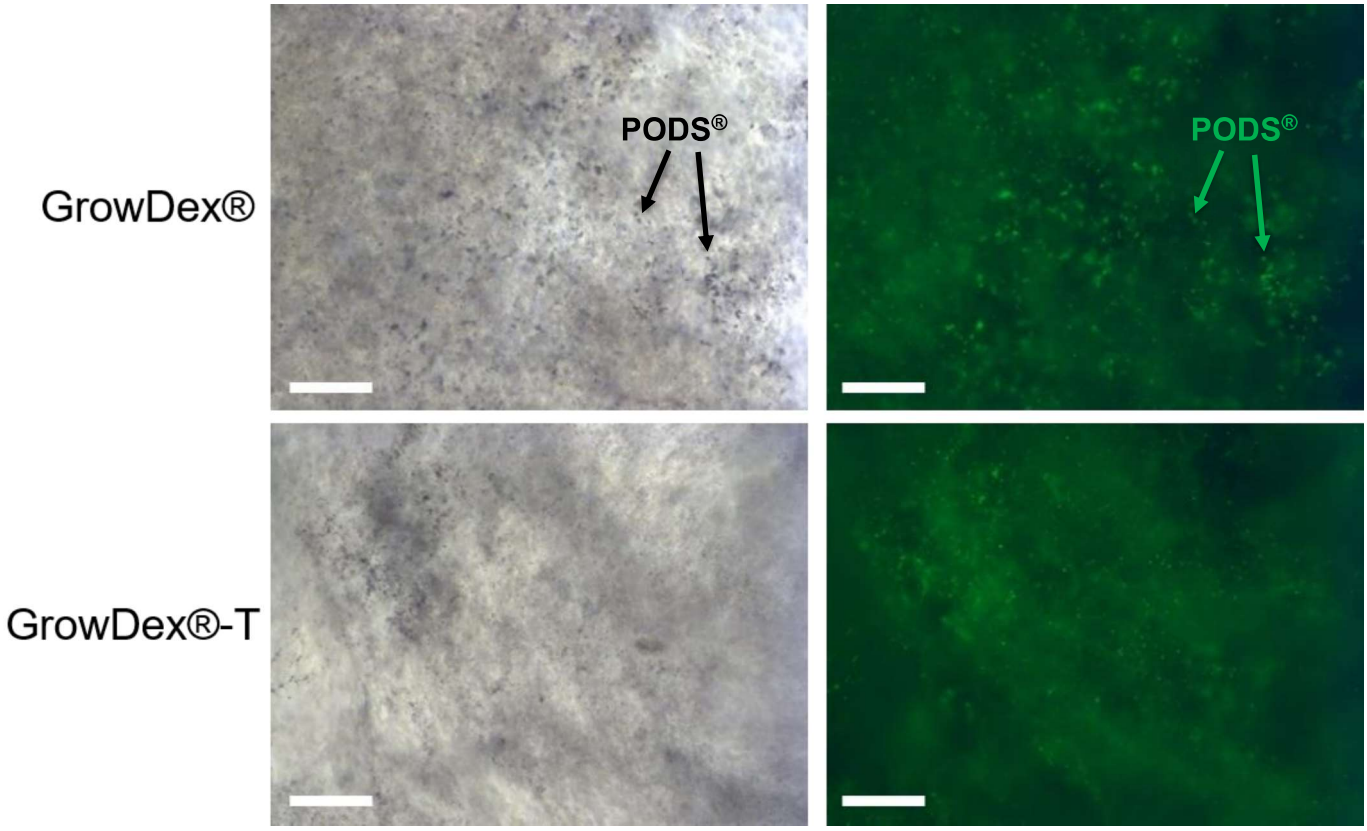


Figure 1. Brightfield and fluorescence images of PODS® GFP embedded in GrowDex® and GrowDex®-T. 5 × 10^5 PODS® GFP were embedded into 50 µl of GrowDex® or GrowDex®-T and cast into a 96-well plate. Note, each fluorescent and black square, respectively, in the entire field of view across all focal planes represents PODS® crystals, as indicated by the arrows. After 3 days of incubation, gels remained intact with PODS® remaining distributed throughout the gel in all planes of view. Scale bar = 200 µm.

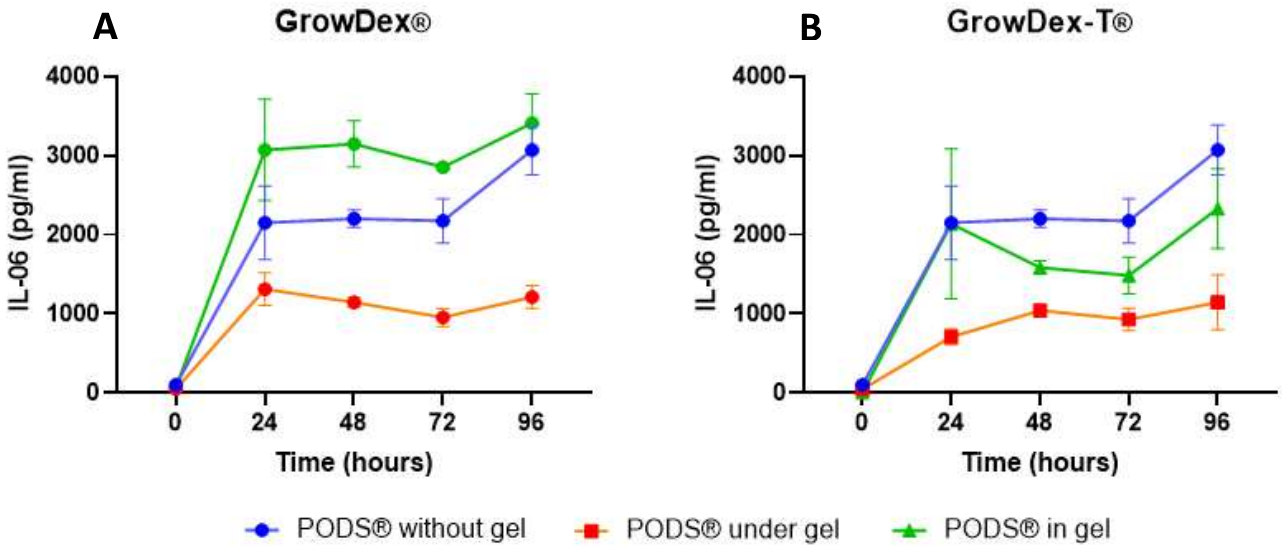


Figure 2. Release of IL-06 from PODS® IL-06 crystals in the presence and absence of GrowDex® (A) and GrowDex®-T (B) incubated with serum-containing media. Release was assessed in 3 conditions. Firstly, a 2D layer of PODS® IL-06 (1 x 10^6) lacking any gel was created by drying crystals onto wells of a 96-well plate. Secondly, GrowDex® gels were cast on top of a 2D PODS® IL-06 crystal layer. Thirdly, PODS® IL-06 crystals were embedded into GrowDex® gels and cast into wells. Subsequently, DMEM + 10% FBS was added to each experimental setup and plates were incubated at 37°C. Supernatant was removed at indicated time points and IL-06 in the medium was quantified by ELISA. Error bars represent standard deviation calculated from at least 2 technical repeats.

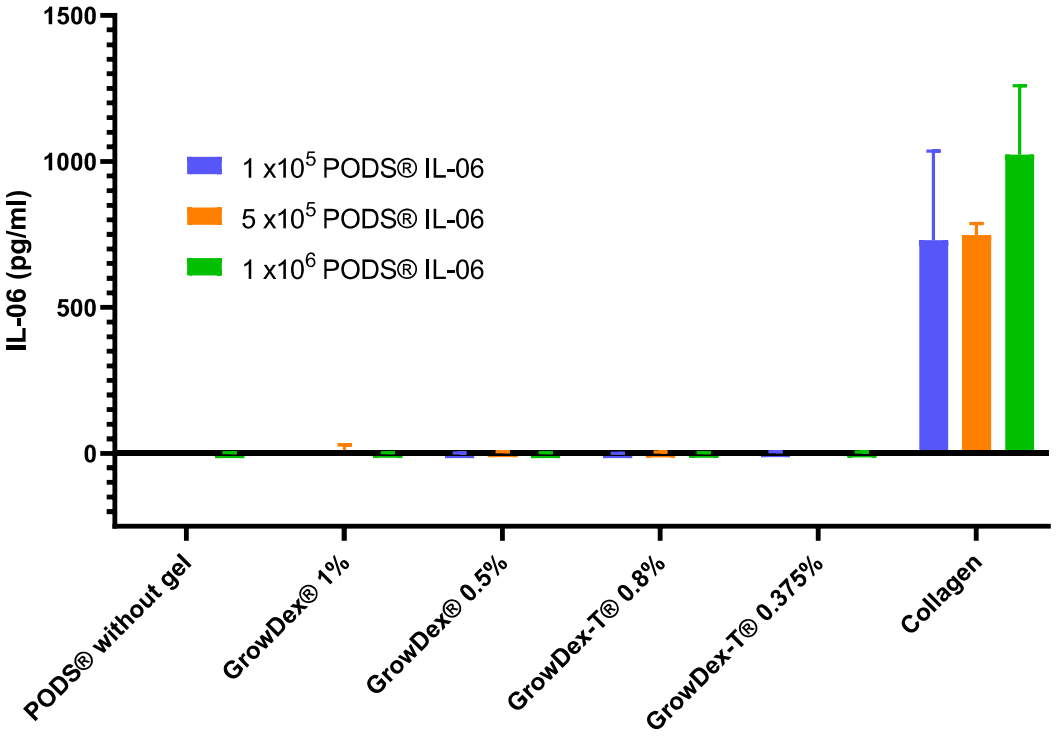


Figure 3. Release of IL-06 from a 2D layer of PODS® IL-06 crystals in the presence and absence of GrowDex® and GrowDex®-T, in serum-free conditions. PODS® IL-06 crystals (1 x 10^5, 5 x 10^5, and 1 x 10^6 per well) were dried onto 96-well plates. Subsequently, either DMEM was added to each well (far left); or gel type as indicated was cast on top of the crystal layer and DMEM was added. The plate was then incubated at 37°C. Supernatant was collected after 3 days and IL-06 concentration in the medium was quantified by ELISA. Error bars represent standard deviation calculated from at least 2 technical repeats.

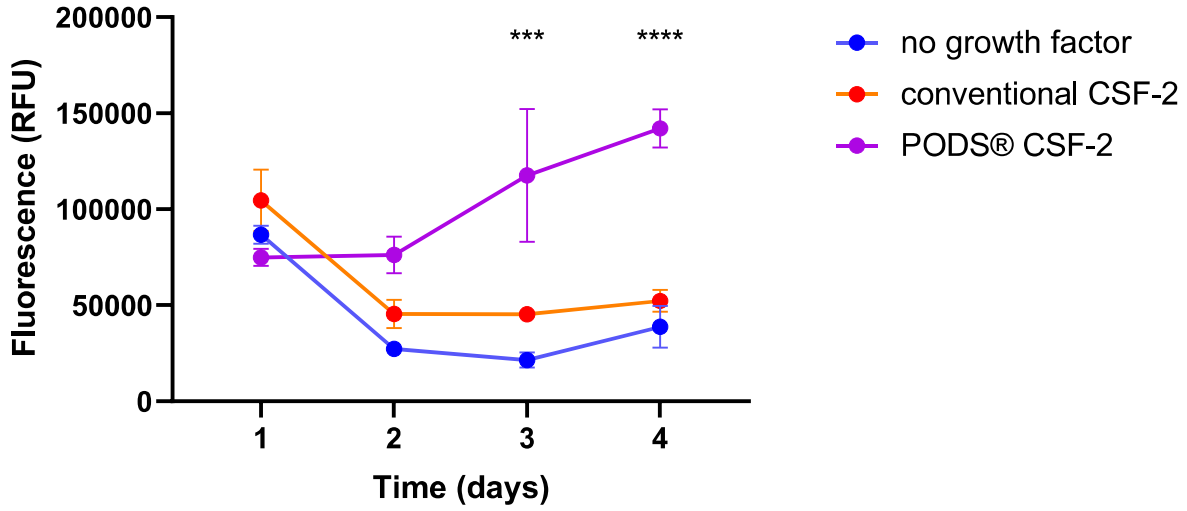


Figure 4. Survival of TF-1 cells when embedded in GrowDex®-T both with and without PODS® GM-CSF (CSF-2) crystals. TF-1 cells were embedded into GrowDex®-T gels, either without growth factor, with conventional soluble CSF-2, or with PODS® CSF-2. Cell activity was measured using PrestoBlue every 24 hours as indicated. Error bars represent standard error of the mean calculated from at least 3 technical repeats.

Conclusions

- PODS® can be easily embedded into Growdex® gels; their distribution and gel integrity are maintained in both short-term and long-term cultures.
- Release of PODS® cargo from GrowDex® gels does not occur in serum-free conditions.
- PODS® in combination with GrowDex® gels release growth factor in a dose-dependent manner in the presence of serum.
- PODS® CSF-2 functionalized GrowDex® gels maintain TF-1 cell survival in culture, outperforming conventional CSF-2.

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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