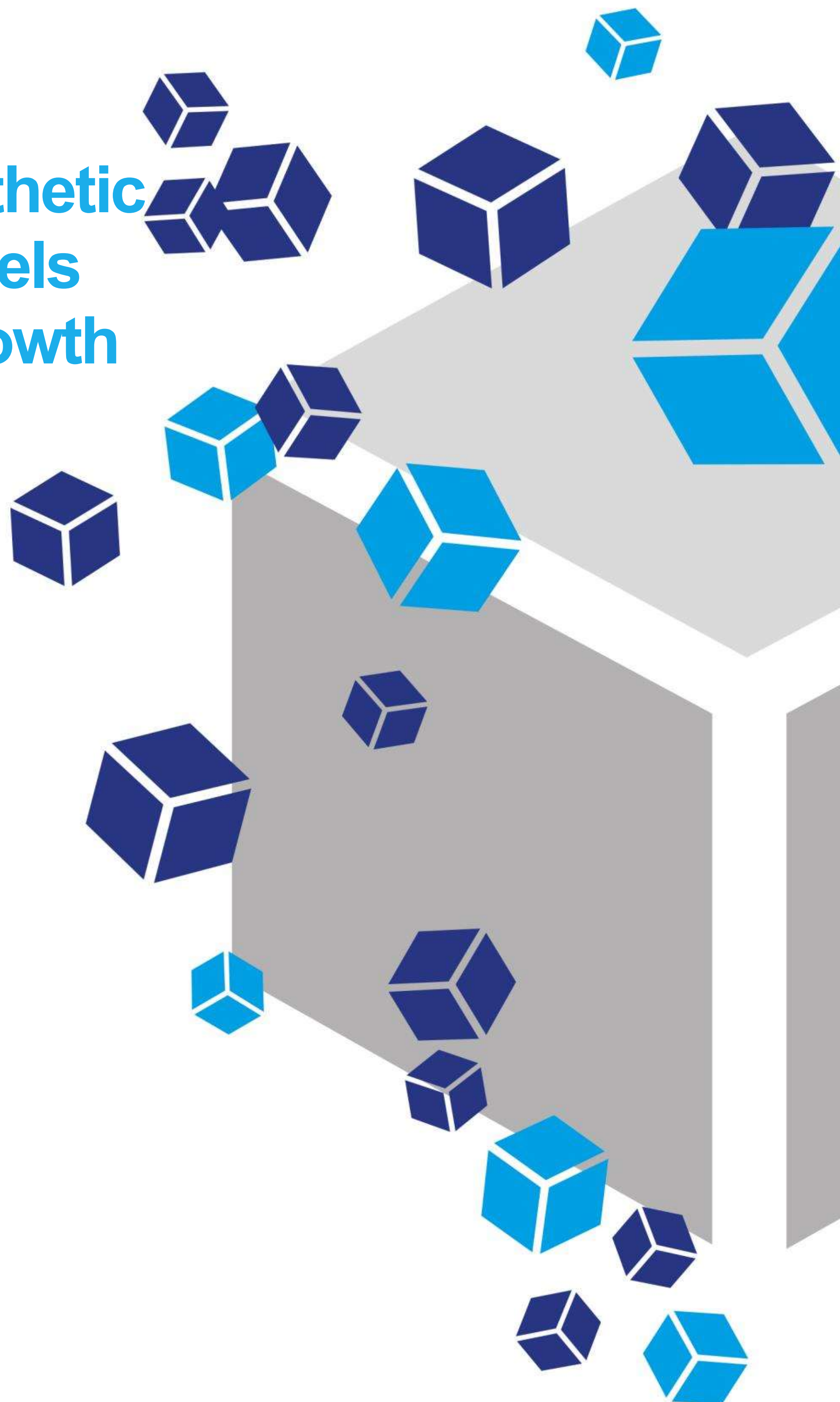
CELL guidance systems

Technical Note

Functionalizing PeptiGels® Synthetic Peptide Hydrogels with PODS® Growth Factors



Functionalizing PeptiGels® synthetic peptide gels PODS® with growth factors

(Issued April 2022)

Introduction to PeptiGels® and PODS®

The challenge with synthetic hydrogels and soluble growth factors

Many synthetic hydrogels are not bioactive. Hence, they need to be functionalised with several proteins and growth factors to initiate bioactivity. On the other hand, 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

The development of a bioactive hydrogel relies on the use 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 PeptiGels®

PeptiGels® are fully synthetic gels composed of oligo-peptides that self-assemble into 3D fibrillar hydrogels. Properties such as the mechanical strength and bio-functionality can be finely tuned to the application by choosing the amino acid composition of the peptides. As such, they are inherently biocompatible and provide a suitable environment for cells to survive and thrive. As synthetic gels, they are also:

- Reproducible
- Transparent
- Animal-free
- Modular
- Ready to use (no temperature requirement)

They also do not require a crosslinker for gel formation, meaning lower toxicity to cells and to the user. Hence, they have multiple advantages over natural hydrogels for 3D cell culture.

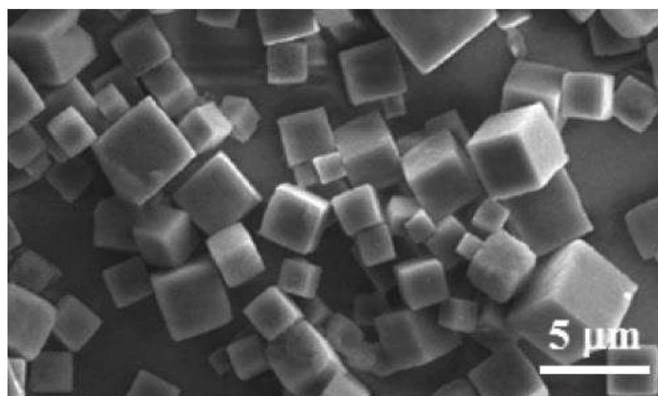
However, providing cells with growth factors and nutritional requirements remains a challenge when using synthetic gels. Combining them with PODS® allows easy embedding and localisation of growth factors within the scaffold, enhancing cell survival, behaviour, and control.

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.



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 this is facilitated by application of a hydrogel into which cells can be embedded, either as a dispersed population or as spheroids or organoids. While animal-derived hydrogels are still commonly used, these suffer from several drawbacks including batch-to-batch variability and that they are chemically ill-defined. The PeptiGel® range from Manchester BioGel are fully defined, synthetic peptide hydrogels. As such, they are inherently biocompatible and biodegradeable, without batch-to-batch variability, animal and disease-free, and thus improve on the reliability and consistency of results over aforementioned animal-derived matrices.

Functionalizing PeptiGels® 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 PeptiGels®, 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 the protocol. 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 (PeptiGel®, Manchester BioGel) were cast into the wells of the TC multi-well plate above the dried PODS® crystal monolayer. Briefly, PeptiGel® 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 gel was dispensed into each well above the PODS® layer. The plate was incubated at 37 °C for 30 minutes, then 100 µl 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 PeptiGel® 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-PeptiGel mix was dispensed into each well of a 96-well plate. The plate was incubated at 37 °C for 30 minutes, then 100 µl media was added on top of each gel.

ELISA analysis: Following collection of samples for analysis, ELISA assays (SEKB10395, SEK10015, SinoBiological) were performed according to the manufacturer's instructions to measure the levels of growth factor in the supernatant.

Results

Distribution of PODS® in PeptiGels®

PODS-PeptiGels were 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).

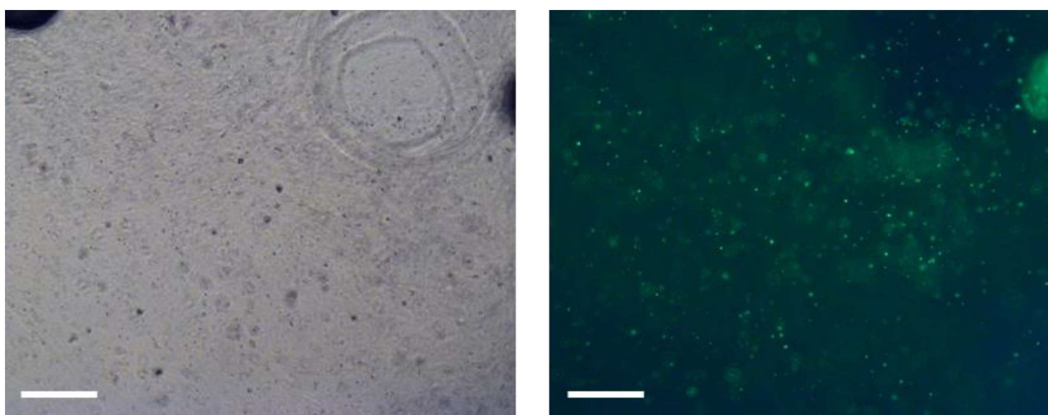


Figure 1. Brightfield and fluorescence images of PODS® GFP embedded in Alpha1 PeptiGel®. 5×10^5 PODS® GFP were embedded into 50 µl of Alpha1 PeptiGel® and cast into a 96-well plate. After 3 days of incubation, gels remained intact with PODS® remaining distributed throughout the gel in all planes of view. Scale bar = 200 µm.

Time-dependent sustained release of PODS® GF in PeptiGels®

The release characteristics of cargo protein from PODS® were compared between different experimental conditions, either PODS® without gel; or within gel (encapsulated in the gel). Release of GF from PODS® crystals could be modulated by varying the placement of PODS® crystals (Fig. 2). The encapsulated PODS® crystals showed a sustained release of GFs for the duration of study. **Also, there was no significant difference between the rate of release of PODS® encapsulated in the gel and without the gel**.

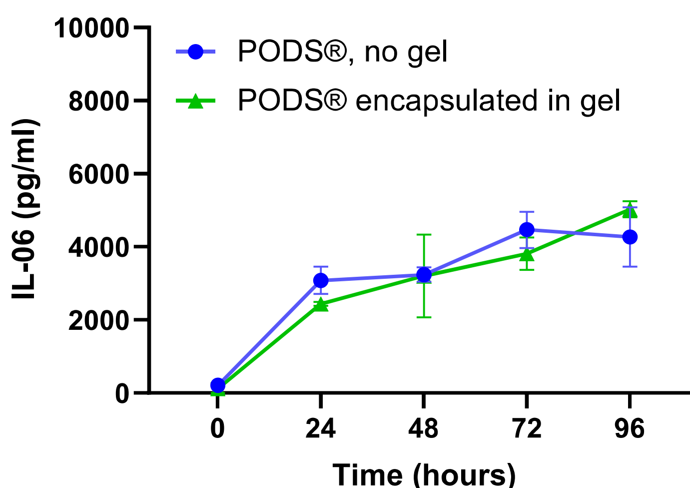


Figure 2. Release of IL-06 from PODS® IL-06 crystals in the presence and absence of PeptiGel® incubated with serum-containing media. Release was assessed under 2 conditions. Firstly, a 2D layer of PODS® IL-06 (1 x 10^6) was created by drying crystals onto wells of a 96-well plate. Secondly, PODS® IL-06 crystals were embedded into Alpha4 PeptiGel® 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.

PODS® compatibility with different PeptiGel® formulations in serum-free conditions

PeptiGels® are available in a variety of formulations with different stiffness and charges, as described in Table 1.

PeptiGel®	Stiffness: G' Post Media (kPa)	Charge
Gamma 1	2.5	Neutral
Alpha 2	8	Positive
Alpha 4	1	Positive

Table 1. PeptiGel® properties.

Release from PODS® could be detected using ELISA in both serum-containing (Fig. 2) and serum-free conditions, the latter demonstrating some differences in release depending on the PeptiGel® formulation chosen (Fig. 3).

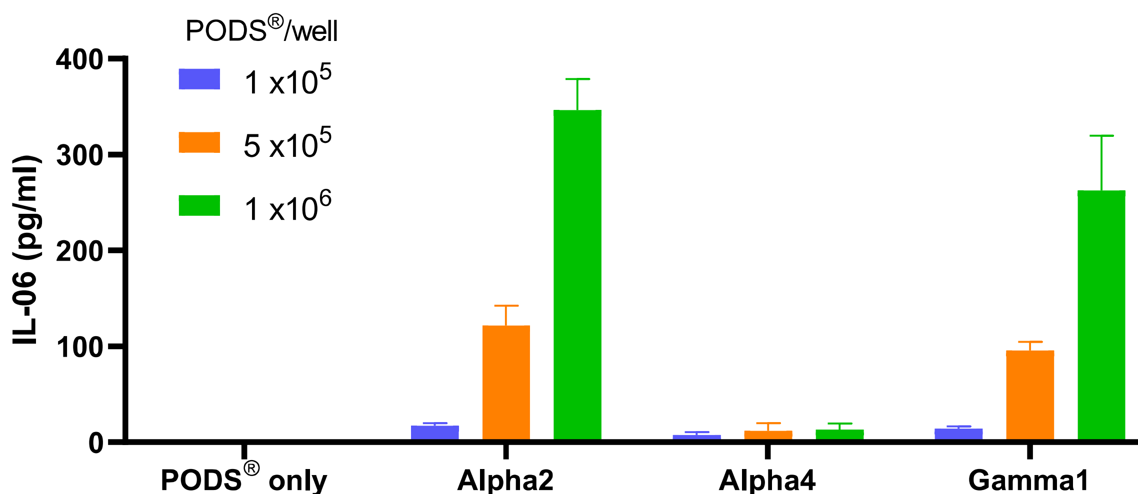


Figure 3. Release of IL-06 from a 2D layer of PODS® IL-06 crystals in the presence and absence of several different types of gel, in serum-free conditions. PODS® IL-06 crystals (1 x 10^5, 5 x 10^5, and 1 x 10^6 per well) were dried onto 96-well plates. Subsequently, either DMEM was added to each well (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.

Impact of mechanical stiffness on release rates

PODS® release rates were not affected by changing the stiffness of a particular gel (Fig. 4). Detected growth factor concentration did not vary when high stiffness (Kappa4), medium stiffness (Alpha 1) and low stiffness (Gamma 4) gels of the same formulation were evaluated with PODS® for growth factor release. These gels have the same chemical composition and differ only in stiffness.

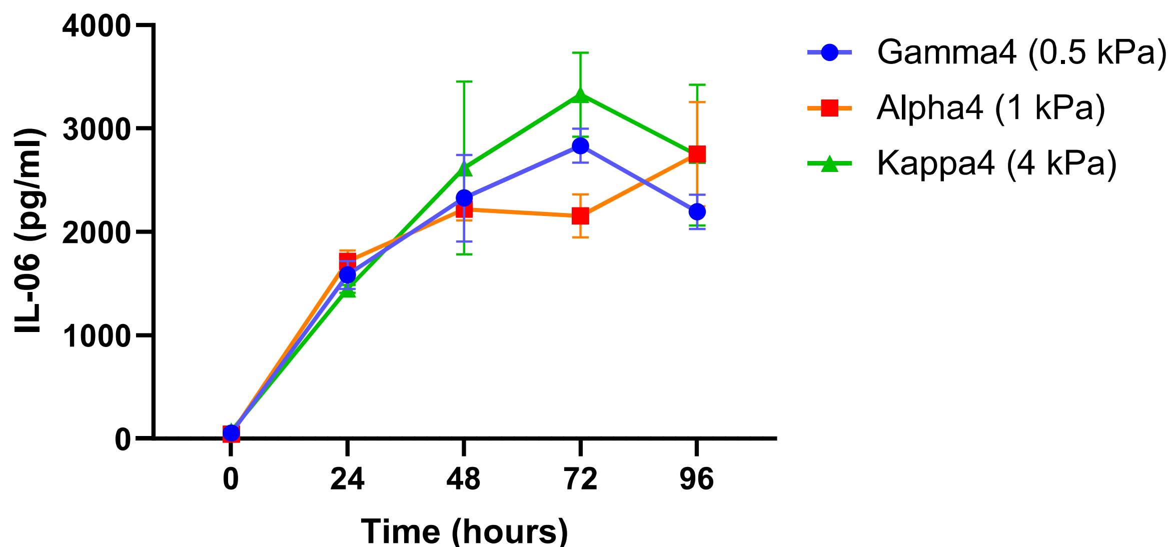


Figure 4. Release of IL-06 from a 2D layer of PODS® IL-06 crystals in PeptiGels® of different stiffnesses. PODS® IL-06 crystals (1 x 10^6 per well) were dried onto 96-well plates. Subsequently, gel was cast on top of the crystal layer and DMEM was added. The plate was then incubated at 37°C. Supernatant was collected at the indicated time points and IL-06 concentration in the medium was quantified by ELISA. Error bars represent standard deviation calculated from at least 2 technical repeats.

Conclusions

- PODS® can be easily embedded into PeptiGels®; their distribution and gel integrity are maintained in both short-term and long-term cultures.
- Release of PODS® cargo from PeptiGels® in serum-free conditions varies depending on the gel formulation used.
- PeptiGel® allows release of PODS® cargo in serum-free or serum-containing cultures.
- PODS® growth factors release cargo of GFs in multiple gel types. For 3D cultures, different PeptiGel® formulation can allow tuning of growth factor release levels and rates.
- Stiffness of gel does not have an impact on growth factor release rates: Alpha4, Gamma4, and Kappa4 all give very similar release rates. These gels have the same chemical composition and differ only in stiffness.

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Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*

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- Instant Exosomes™ - purified and characterized
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