Frequently Asked Questions (FAQs)

PODS-PeptiGels

Sustained Release Growth Factors and Synthetic Peptide Hydrogels

Version 1.0

PODS-PeptiGels

PODS® FAQs

- **How big are PODS® nanoparticles?**

PODS® crystals are cubic and typically 0.2-5 microns in size with a modal size of 1-2 microns.

- **What types of molecules can you put in PODS® nanoparticles?**

PODS® can be used with protein cargos. Large proteins can be loaded. Nucleic acids may be possible. Small molecules are not suitable.

- **What is a PODS® nanoparticle's buoyant density?**

PODS® co-crystals are slightly heavier than water and will settle on the surface of a culture vessel. Care should be taken when aliquoting since PODS® crystals will sink to the bottom of a tube within a few minutes. The majority of PODS® crystals will remain in suspension for up to 60 min in a 20% glucose solution (or a solution of similar density).

- **How stable are PODS® nanoparticles?**

We have tested a variety of conditions to assess their impact on the integrity of PODS® crystals. PODS® crystals are stable at high temperatures for short periods. They are highly stable when stored in solutions between pH 6-8 and can withstand pH 3-10. Above pH 10, PODS® crystals lose their stability and dissolve rapidly (in a few hours). PODS® crystals are stable in standard cell culturing temperatures for extended periods of time (>6 months).

- **How is the active protein released?**

PODS® protein cargo is released by proteases. Proteases may be derived from components of the solution (e.g. serum) or secreted by nearby cells. Therefore, the culture system affects the amount of growth factor available in solution. In contrast to gel-encapsulated proteins (using hydrogels such as PLGA) PODS® crystals do not produce an initial burst release of cargo proteins. For naked PODS® crystals in cell culture, peak release in culture systems typically occur at day 2, then gradually diminishes. Peak-availability is not the same as peak-release-rate and depends on a number of factors including the rate of protease released by cells and the inherent stability (half-life) of the cargo protein.

- **Is it possible to modify the release profile?**

The release of active proteins into cell culture occurs over a period of 1-3 weeks. This release period can be extended if the PODS® crystals are combined with a scaffold, which acts as a barrier to protease activity. Release rates will also be influenced by the presence of proteases in the scaffold. Bovine collagens, for example, will contain proteases.

- **Why are PODS® sold by numbers of particles?**

PODS® active proteins are incorporated into the polyhedrin crystal within the insect cell. Consequently, it is not possible to determine the amount of active protein that is present in a sample by measuring optical density. When PODS® crystals are expressed, both polyhedrin proteins and active proteins are expressed from within a single vector and both utilize the same promoter sequence. Therefore, the ratio of active to polyhedrin protein will be constant. The size distribution of PODS® crystals is also constant. Consequently, the number of crystals has been adopted as the unit of quantification for PODS®. It has been estimated that $50x10^6$ PODS® crystals typically generates a peak concentration in the medium equivalent to 3.3 µg of standard recombinant protein.

- **How many PODS® nanoparticles should be used?**

PODS® crystals provide a depot of active proteins which are steadily secreted. As stated above, in one experiment it has been estimated that the biological activity of $50x10^6$ PODS® crystals generates the same peak dose as 3.3 µg of standard recombinant protein in a LIF-dependent mouse ESC culture system. However, at 5 days following the start of seeding the PODS® crystals, more than 50% of these peak levels are still present in the culture system. Ultimately, the amount of PODS® crystals that are optimal for a particular experiment should be determined empirically, using $50x\ 10^6$ PODS® crystals equivalence to 3.3 µg of standard growth factor as a good starting point.

- **How often should media and PODS® nanoparticles be replaced in the culture system?**

The frequency of media change depends on (1) the speed with which nutrients are exhausted or degraded and (2) the speed with which toxic metabolites accumulate. In most cell culture systems, the stability of growth factors is an over-riding issue which drives media replenishment. However, particularly in cells with high levels of metabolic activity, other factors will eventually become important once the stability issue has been addressed. We have maintained growth-factor-dependent cultures for several weeks without needing to add new PODS® crystals.

- **Are PODS® nanoparticles transparent?**

PODS® without any cargo are isotropic crystals that do not refract light. However, any cargo protein impacts on this property such that they will refract light. PODS® crystals generally do not interfere with the generation of images using imaging techniques such as phase-contrast and may also interfere with an assay that utilizes the measurement of optical density.

- **Can PODS® crystals have a physical impact on cell behaviour?**

Physical features on a culture surface may impact the behaviour of certain cells, particularly at high densities. If PODS® crystal topology is a concern, PODS® crystals may simply be incorporated into a hydrogel surface coating. Phagocytic cells (such as macrophages) will ingest PODS®. We have not observed any effect on the health of these cells. However, if this is a concern, the PODS crystals may be incorporated into a gel.

- **Do PODS® cargo proteins have post-translational modifications?**

PODS® crystals are made in insect cells. PODS® active proteins, therefore, contain most of the post-translational modifications that are also found in mammalian cells.

- **Are PODS® nanoparticles immunogenic?**

The polyhedrin protein has been tested many times in-vivo and, although antibodies are generated against polyhedrin, an obvious inflammatory response has not been observed. Lack of an inflammatory response for foreign proteins is not without precedent: silk fibroin protein from the silkworm *B. mori* is commonly used for surgical stitching and Botulinum toxin (Botox) is widely injected in cosmetic procedures.

- **Are PODS® proteins equivalent to standard growth factors?**

The stability of standard recombinant growth factors varies significantly. The most labile, such as FGF-1, have a sera half-life measured in minutes limiting their utility. The stability of PODS® growth factors that are encased in PODS® crystals is much longer but once a molecule is released will have the same half-life as their standard recombinant counterparts. This may be advantageous for 3D applications as it reduces the path length

The amount of available growth factor in a culture system is a function of the speed of release from the crystal and the subsequent stability. In the first few hours of a culture containing only newly added PODS® crystals, there is little growth factor protein available for the cells. Significant amounts of growth factor protein are available after one day. The initial lag of protein availability may be corrected if necessary, by adding a small amount of standard recombinant growth factor

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PeptiGels® FAQs

- **What is the mechanical strength and charges of PeptiGels®?**

PeptiGels® have a range of mechanical stiffness and charges to allow you find the most suitable environment for your cells' needs. We also offer a bespoke design service to create PeptiGels® specific to your research needs.

- **Can you dilute PeptiGels®?**

You can dilute PeptiGels® with our specially formulated, ready to use solution, PeptiSol®, which is specifically designed to help you achieve your desired mechanical strength/s. We also supply specific mechanical strength PeptiGels® as required.

- **Do you need to work on ice when you work with PeptiGels®?**

You do not need to work on ice while working with PeptiGels® as they are stable at room temperature.

- **What is the shelf life of PeptiGels®?**

Our PeptiGels® are stable for 12 months once open.

- **What are the storage conditions on PeptiGels®?**

We recommend you store the PeptiGels® at 4°c when you're not using them.

- **How does the gelation process of PeptiGels® work?**

PeptiGels® have been designed to gel at room temperature by the addition of cell culture media (contains salts). The ionic salts present in the media screen the charges present on the peptide fibres which causes them to aggregate and induce gelation.

- **How easily can you pipette PeptiGels®?**

PeptiGels® are low viscosity gels and can be pipetted easily using positive displacement pipettes. Get in touch if you need one of these.

- **Can cells migrate in PeptiGels®?**

PeptiGels® are nanofibrous porous hydrogels. Cells are able to migrate within the hydrogel making them also suitable for migration-based assays.

- **What is the maximum duration of time for cells cultured in PeptiGels®?**

The maximum duration of cells in PeptiGels® culture is dependent on the cell type and experimental set up. Cells have been cultured in PeptiGels® up to 30 days. We have specific protocols to guide you towards cell culture and long stability of the gels. Please see our protocols webpage for further details.

- **What cell density do you recommend for PeptiGels®?**

PeptiGels® are suitable for a broad range of cell densities from 1–40 million cells/mL in various studies. The cell density needed is usually dependent on your experimental set up and intended outcomes. As a guide for 2D cultures, a cell seeding density comparable to that used on standard tissue culture is recommended. For 3D cultures, the most commonly used cell density is 1-4 million cells/mL.

- **What applications are suitable for the use of PeptiGels®?**

PeptiGels® have been shown to support the growth of cells in 2-dimensions, 3-dimensions, in co-culture systems and into spheroids and organoids. These have been used for tissue and disease modelling, regenerative medicine applications, vehicles for the targeted and controlled delivery of therapeutics and also incorporation within medical devices. A full range of cells have been studied ranging from cardiac to skin to bone and also a full range of animal and human derived stem cells for proliferation and differentiation.

- **What cell types are suitable for PeptiGels®?**

PeptiGels® have been shown to support and promote the growth of an ever-expanding list of cells. Examples include primary cells such as fibroblasts, neuronal cells and endothelial cells, transformed cell lines such as MCF-7 cells and stem cells such as induced pluripotent stem cells and mesenchymal stem cells.

- **Are PeptiGels® compatible with end-point molecular analysis of proteins and nucleic acids?**

PeptiGels® are compatible with most molecular techniques such as quantitative polymerase chain reaction (q-PCR), western immunoblotting and proteomic analysis. Please see our detailed protocols on sample preparation for these analyses.

- **Are PeptiGels® compatible with staining procedures?**

Cells cultured in PeptiGels® can be stained with fluorescent dyes and immunological reagents. Please see detailed protocols on sample preparation for staining procedures.

- **Are PeptiGels® compatible with microscopy?**

PeptiGels® are transparent gels, hence compatible with a diverse range of microscopic techniques including optical, fluorescent and confocal microscopes.

PODS-PeptiGels FAQs

- **How many PODS® should I use per ml of PeptiGel®?**

The appropriate number of PODS® for your experimental system should be empirically determined using a range of PODS® numbers. We recommend a starting point between 2 million to 20 million PODS® per ml of gel. The amounts of PODS® cargo released will depend on the stability of the growth factor used and the PeptiGel® selected. Biological effects of PODS® will also vary depending on your cell type and desired readouts.

- **Which PeptiGel® should I use in my system?**

A range of PeptiGels® is available with different properties such as stiffness and charge are available to mimic the extracellular matrix of different tissues and present different adhesion motifs. The most suitable gel will depend on your cell type and the aims of your experiments. Please get in contact to find out more.

- **Should I use PODS® embedded in PeptiGel® or underneath PeptiGel®?**

PODS® cargo will be released and diffuse throughout PeptiGel® regardless of whether they are placed underneath the gels or are embedded. Placing them underneath will create a gradient in the upwards direction towards which cells may migrate depending on the growth factor. Distributing them throughout the gel will create a more homogenous distribution of growth factor throughout the gel, and increase the proximity of growth factor to cells.

- **Can I use PODS-PeptiGel in a microfluidic device?**

PODS-PeptiGels can be used in microfluidic devices.

- **Can I use PODS-PetiGel for bioprinting?**

PODS-PeptiGels can be used for bioprinting. Consider combining PODS® with PeptiInks® rather than PeptiGels® as these are especially formulated for use with bioprinters. As PODS® provide depots of growth factors they can be localised to initiate gradients of growth factor throughout the gel. please contact us for more information and advice on how to establish a gradient within a 3D printed structure.

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

Matrix Proteins

Small Molecules

Cell Counting Reagent

Cytogenetics Analysis

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