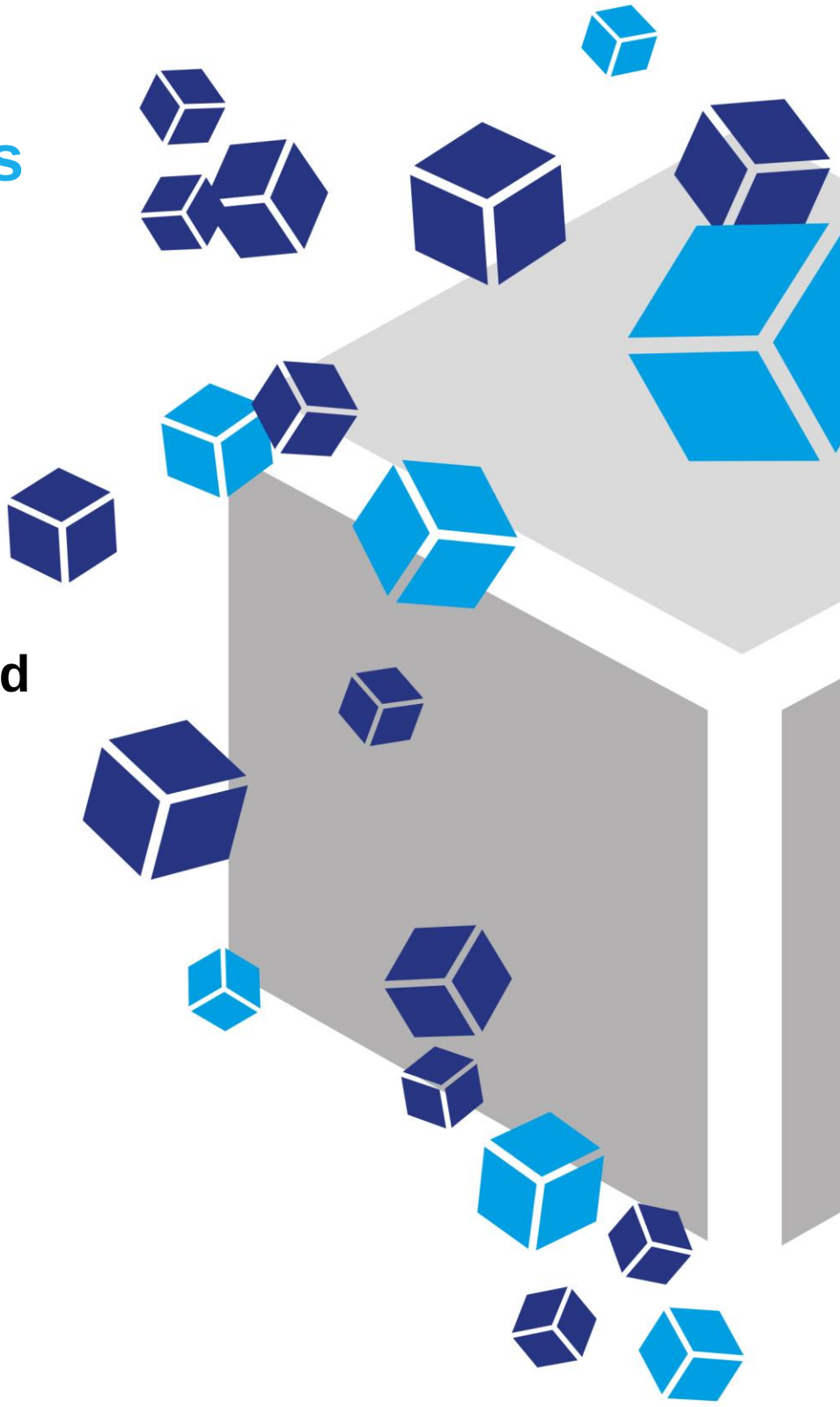
User Guide

PODS-PeptiGels



Sustained Release Growth Factors and Synthetic Peptide Hydrogels

Cat PPH

Protocol Version 1.0

Contents

PODS–PeptiGels

Storage

Upon receipt, store at 4°C.

PODS® crystals and PeptiGels® are stable for at least a year at when stored at 4°C.

Reconstitution

PODS® crystals may be reconstituted at 200x10^6 crystals/ml in water. Alternatively, 20% glucose has a buoyant density closer to PODS® crystals and can be useful for aliquoting. PODS® crystals are highly stable when stored in aqueous solution (pH range 6-8) at 4°C and have been shown to maintain stability for at least 6 months.

Product information

Introduction

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.

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.

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.

POlyhedrin Delivery System (PODS®), based on the polyhedrin protein crystal, provides an effective solution to the inherent instability of proteins. Polyhedrin protein forms regular, cubic crystals containing the protein/growth factor of interest (PODS®) which provide a slow-release depot formulation for the active protein of interest. When combined with PeptiGels®, tuneable self-assembly peptide hydrogels, they can functionalise the scaffold to slowly release the growth factor, delivering growth factor in close proximity to cells and minimising required handling time.

General notes

We highly recommend the use of a positive displacement pipette (such as the Gilson piston pipette) to allow easy pipetting as these are viscous gels.

PeptiGels® should be diluted with PeptiSol prior to casting into the well plate. Dilution required may vary depending on the application and cell type. Recommended dilution is ratio 1:4 of PeptiSol to PeptiGel volumes accordingly.

As a guide, this protocol has been written for a total volume of 50 µL PODS-PeptiGel®. Please scale up or down according to culture requirements.

PODS® preparation

1. Remove PODS from the fridge.

2. Reconstitute the lyophilised PODS® in PBS without calcium and magnesium to make a stock solution of 5 x 10^7 PODS®/ml. For a vial of 50 million PODS®, add 1 ml PBS to make the stock solution.

   Hint: The stock solution will contain approximately 50 million PODS in a total volume of 1mL.

3. Vortex for 30 seconds at 2400 rpm to fully resuspend the pellet.

   Hint: Check to see the PODS are resuspended by the appearance of a milky white solution. PODS® settle quickly and therefore ensure PODS® are resuspended by vortexing immediately before the next step.

4. Aliquot the required number of PODS® from stock solution. The recommended range is 10,000 – 1,000,000 PODS® per 50 µL of gel. (Store the remaining stock solution at 4 °C. Please do not freeze.)

   As a guide, you can start with 100,000 PODS per 50 µL. Please note, further optimisation of PODS® concentration may be required depending on cell type, gel, and experimental aims. Please see table 1 as a guide for PODS® numbers to be used in 50 µL total volume of gel. Please scale up or down according to the total volume required for the experimental design (i.e. given the number of gels required for the experiment). Please note that it is pragmatic to include some excess volume to compensate for material loss during the pipetting process.

5. Spin the aliquot in a centrifuge at 3000 x *g* for 5 minutes to pellet PODS®

6. Following the spin, gently remove all liquid using a pipette and discard the supernatant. The PODS® will be visible as a small white pellet.

If culturing cells in 2D, embed PODS® into gels as below, then seed cells on top. If culturing cells in 3D (embedded within the gel), then proceed directly to that section below.

Table 1. From a stock solution of 50 million PODS® per mL

	For 50 µL gel	For 500 µL gel
PODS® concentration per 50 µL gel	**Volume required from PODS® stock (µL)**	**Volume required from PODS® stock (µL)**
10,000	1	10
100,000	10	100
1,000,000	100	1000

Embedding PODS® into PeptiGels®

1. Resuspend the PODS® pellet in the required amount of media or PeptiSol and vortex (2400 rpm) to mix. As a guide, if making 1 ml of gel, resuspend in 200 µL media (or PeptiSol) i.e. 1/5th of the final volume

   Hint: For a 96-well plate, use 30-50 µL of gel per well. Make up around 30% excess gel compared to the amount required for all samples. For example, if 1 mL total gel is required for all samples, make up 1.3 mL of gel total to compensate for any fluid loss during the pipetting process.

   Hint: PODS® settle quickly and therefore ensure PODS® are resuspended by vortexing immediately before the next step.

2. Using a positive displacement pipette, add the appropriate volume of PeptiGel® to the PODS® in media and pipette up and down to mix thoroughly. As a guide, if preparing 1 ml of gel, add 800 µL of gel to the 200 µL of resuspended PODS® in media.

   Hint: Gently vortexing at this stage, at approximately 800 rpm for 2 seconds, will help to distribute the PODS® throughout the gel.

3. Dispense 50 µL of PODS-PeptiGel into wells of a 96-well plate using a positive displacement pipette.
4. Add 100 µL media to the dispensed gel and incubate at 37 °C and 5% CO2.
5. After 30 minutes of incubation, discard the supernatant.
6. Replace with 100 µL media to the dispensed gel and incubate at 37 °C and 5% CO2 for another 30 minutes.
7. Discard the media and the PODS-PeptiGel will be ready for cell seeding.

2D culture of cells in PODS-PeptiGels

1. Resuspend your cells at the desired concentration in 100 µL media per PODS-PeptiGel (if using a 96-well plate with 50 µL gels: adjust volume as needed for different plate formats).

   Hint: For most cell types, a cell concentration of 1 x 10^6 cells/mL is usually recommended.

2. Remove the 100 µL of media and add your cell suspension carefully to the PODS-PeptiGel in the 96 well plates.
3. Incubate overnight at 37 °C and 5% CO2.
4. Next day, change your media according to the cell requirement.

3D culture of cells in PODS-PeptiGels

1. Prepare a cell pellet containing the desired number of cells for the experiment: centrifuge cells and remove the media. Set the pellet aside whilst performing the next step.

   Hint: 10,000 cells per 50 ul PODS-PeptiGel is a good starting point for most cell types.

2. Resuspend the PODS® pellet in the required amount of media or PeptiSol and vortex (2400 rpm) to mix. As a guide, if making 1 ml of gel, resuspend in 200 µL media (or PeptiSol) i.e. 1/5th of the final volume

   Hint: For a 96-well plate, use 30-50 µL of gel per well. Make up around 30% excess gel compared to the amount required for all samples. For example, if 1 mL total gel is required for all samples, make up 1.3 mL of gel total to compensate for any fluid loss during the pipetting process.

   Hint: PODS® settle quickly and therefore ensure PODS® are resuspended by vortexing immediately before the next step.

3. Transfer the PODS® suspension to the tube containing the cell pellet and use it to resuspend the cells by pipetting up and down. As a guide, transfer 200 ul of PODS suspension for 1 ml of PODS-PeptiGel.
4. Using a positive displacement pipette, add the appropriate volume of PeptiGel® to the PODS® and cells in media and pipette up and down to mix thoroughly. As a guide, if preparing 1 ml of gel, add 800 µL of gel to the 200 µL of resuspended PODS® and cells in media.

   Hint: Gently vortexing at this stage, at 800 rpm for 2 seconds will help to distribute the PODS® throughout the gel.

5. Dispense 50 µL of PODS-PeptiGel containing cells into wells of a 96-well plate using a positive displacement pipette.
6. Add 100 µL media to each gel and place into the incubator at 37 °C and 5% CO2.
7. After 30 minutes of incubation, replace with 100 ul fresh media and put back into an incubator for another 30 minutes.
8. Incubate overnight at 37 °C and 5% CO2.
9. Next day, change the media according to cell requirements.

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General info@cellgs.com
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www.cellgs.com

EUROPE
Cell Guidance Systems Ltd
Maia Building
Babraham Bioscience Campus
Cambridge
CB22 3AT
United Kingdom
T +44 (0) 1223 967316
F +44 (0) 1223 750186

USA
Cell Guidance Systems LLC
Helix Center
1100 Corporate Square Drive
St. Louis
MO 63132
USA
T 760 450 4304
F 314 485 5424

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Mereside, Alderley Park, Alderley Edge, Cheshire SK10 4TG
t: +44 (0) 1625 238800
e: info@manchesterbiogel.com

www.manchesterbiogel.com