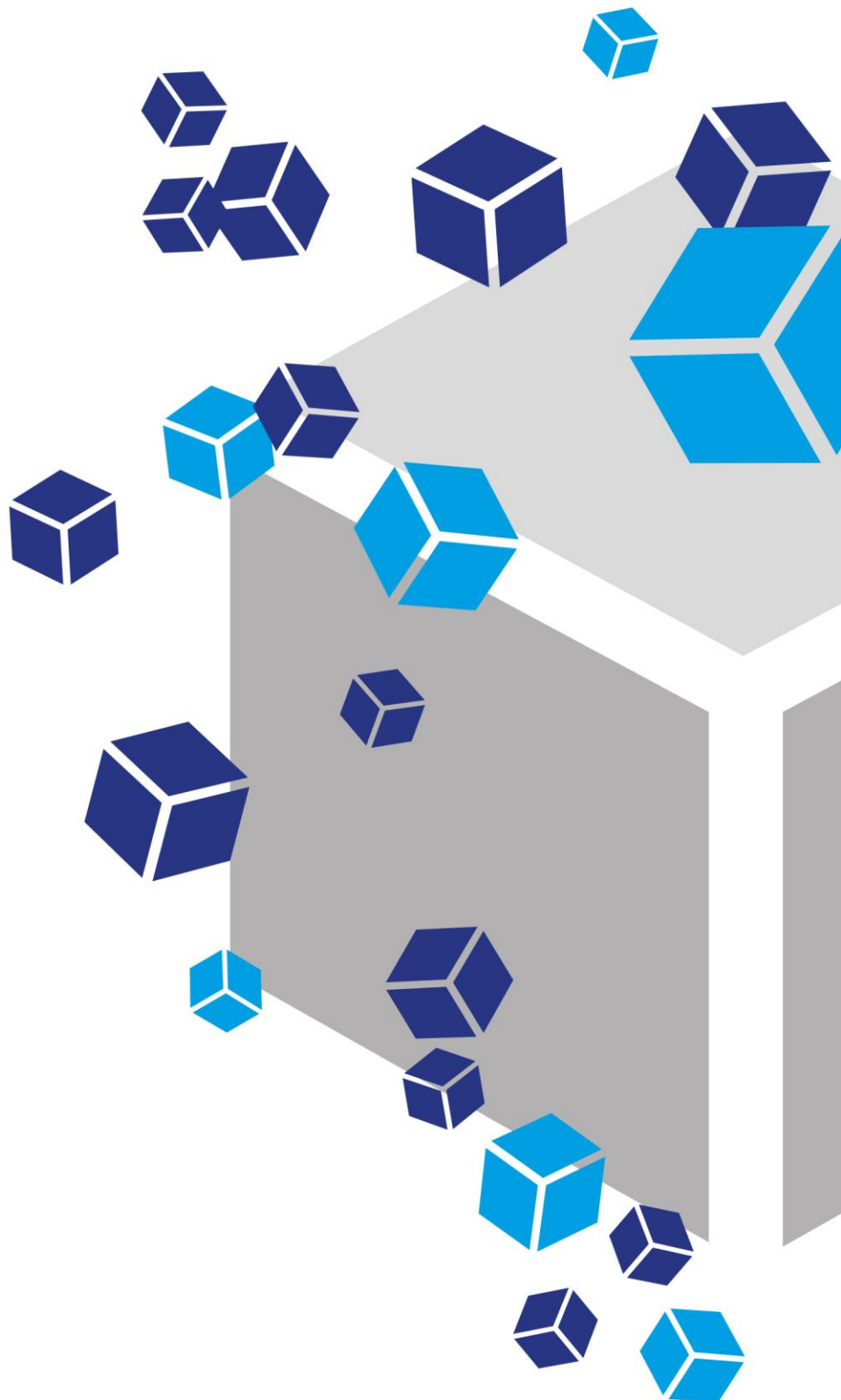


Application Note

Use of PODS- PeptiGels for complex 3D *in vitro* culture



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(Issued February 2021)

Introduction to PODS-PeptiGels

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.

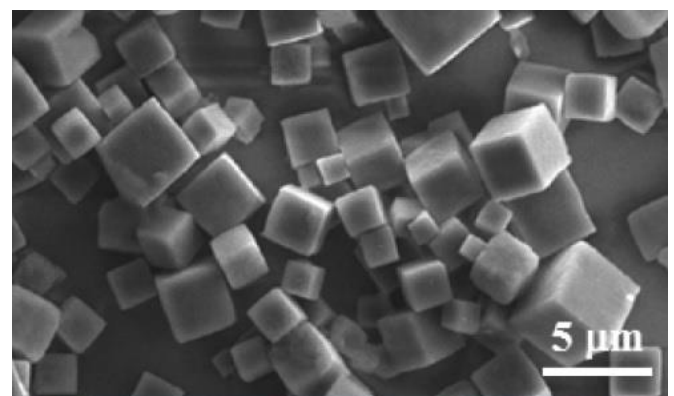
However, providing cells with growth factors and nutritional requirements remains a challenge when using synthetic gels. Combining them with PODS® allows easy embedding and localisation of growth factors within the scaffold, enhancing cell survival, behaviour, and control.

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. And as such they are inherently biocompatible and biodegradable, without batch-to-batch variability, animal and disease-free and thus improve on the reliability and consistency of results over aforementioned animal-derived matrices.

The addition of PODS® growth factors to culture systems using PeptiGels® can functionalize an otherwise biologically inactive material, providing depots of growth factor (GF) that will release over an extended period of time. Conventional GFs can be very labile and degrade quickly in culture, diminishing their usefulness, and thus requiring constant replenishment. Embedded PODS® can reduce the handling frequency, something which is particularly useful for complex cultures that require minimum disturbance, whilst also reducing cost. This study demonstrates that PODS®-functionalized PeptiGels® promote cell survival and proliferation above and beyond what can be achieved with conventional growth factors.

Methods

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 pipetted up and down to resuspend. The remaining volume (4/5) of PeptiGel® was added to this suspension and the PODS® were mixed throughout by pipetting with a positive displacement pipette. 30 µl 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.

Embedding cells into PeptiGels®: NIH 3T3 fibroblasts were embedded into PeptiGels® by adding them into the media portion of the final gel volume using the same procedure as for PODS® described above, at a cell density adjusted to produce 12,000 cells per 30 µl of gel.

Cell number analysis: Following the appropriate incubation period, gels were digested with 10 mg/ml [pronase](#) (Sigma, PRON-RO; 70 µl pronase to 30 µl gel) for 1 hour at 37 °C, agitating the gel periodically with a pipette tip to ensure complete digestion. Following the digestion, 120 µl of 2X TE buffer containing 1% Triton was added to the samples, mixed with gentle pipetting and incubated for 30 minutes at 37 °C. Samples were then stored at -20°C for a minimum of 30 minutes or until ready to quantify. The samples were thawed, then 100 µl of sample in duplicate was added to the wells of a black-walled 96-well plate. 100 µl of [PicoGreen®](#) dye (ThermoFisher, P7589), diluted 1:200 in 2X TE buffer, was then added to each sample. Mixing was achieved by placing the plate on a shaker for 1 minute. The DNA content was quantified by measuring fluorescence at excitation and emission wavelengths of 480 nm and 520 nm, respectively. Fluorescence values were translated into cell numbers using a standard curve of serial dilutions of cells from a known concentration.

Cell staining: Cell cultures were fixed in 4% paraformaldehyde for 15 minutes at appropriate time points. They were washed in PBS three times, then samples were permeabilized in PBS containing 1% Triton X for 1 hour at 37 °C. Samples were once again washed in PBS, then 1:1000 dilutions of both [Hoechst nuclear stain](#) (ThermoFisher, H1399) and [rhodamine phalloidin](#) (Abcam, ab235138) in PBS with 1% BSA were added. The samples were incubated for 90 minutes, washed again 3x with PBS, then imaged on a fluorescence microscope.

Results

Biocompatibility of PODS-PeptiGels with 3T3 fibroblasts

Murine fibroblasts (NIH 3T3) were cultured within PeptiGel[®] both with and without PODS[®] FGF-2, and also compared with conventional (soluble) FGF-2. Only cells cultured with PODS[®] FGF-2 displayed an increased spreading behavior and normal fibroblast morphology (Fig. 1)

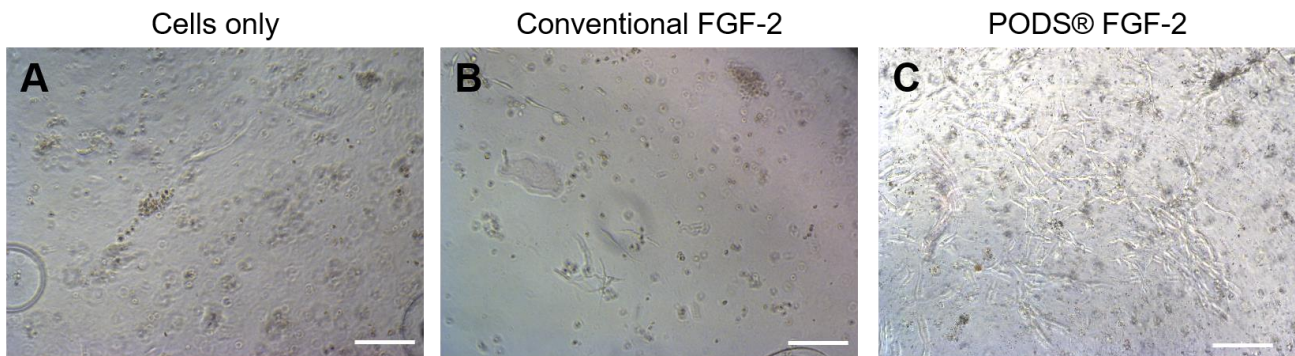


Figure 1. Brightfield microscopy images of NIH 3T3 mouse fibroblasts embedded in Gamma2 PeptiGel[®]. Cells were embedded into PeptiGel[®] and cultured in complete medium either without supplement (A), supplemented with conventional FGF-2 (B), or with gel functionalized with PODS[®] FGF-2 (C). They were imaged after 4 days in culture. Media used was DMEM with pyruvate and supplemented with 10% BCS. Scale bar = 200 μ m.

Characterisation of cells cultured in PODS-PeptiGels

Murine fibroblasts (NIH 3T3) were cultured within PeptiGel[®] both with and without PODS[®] FGF-2, and also compared with conventional (soluble) FGF-2. Cells in PODS-FGF2 showed enhanced fibroblastic morphology as observed by staining of the cytoskeleton (Fig. 2).

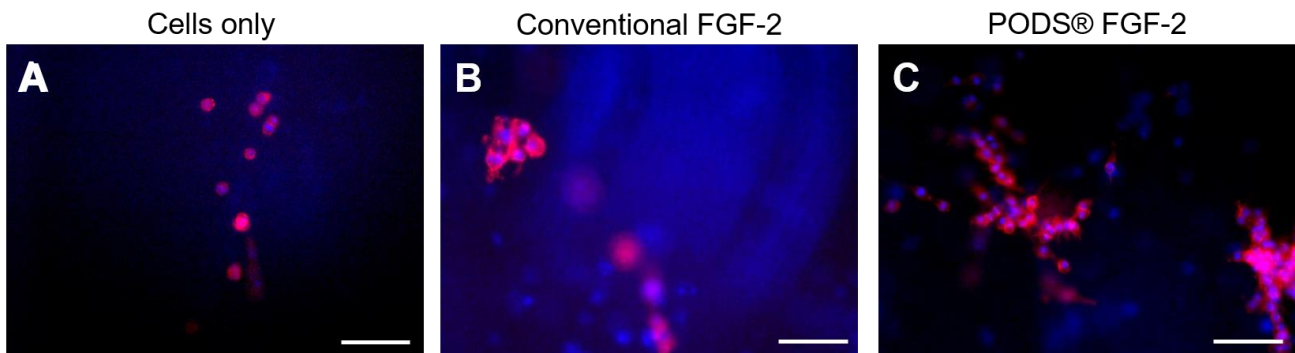


Figure 2. Fluorescence microscopy images of NIH 3T3 mouse fibroblasts embedded in Gamma2 PeptiGel[®]. Cells were embedded into PeptiGel[®] and cultured in complete medium either without supplement (A), supplemented with conventional FGF-2 (B), or with gel functionalized with PODS[®] FGF-2 (C). They were fixed, stained, and imaged after 7 days in culture. Blue, Hoechst nuclear stain; red, rhodamine phalloidin (actin). Scale bar = 100 μ m.

Comparative Proliferation rates of PODS® GFs vs. Conventional GFs

Furthermore, PODS® FGF-2 functionalized PeptiGel® achieved an increased level of proliferation over a period of 7 days following seeding, as measured by DNA content in the gels (Fig. 3).

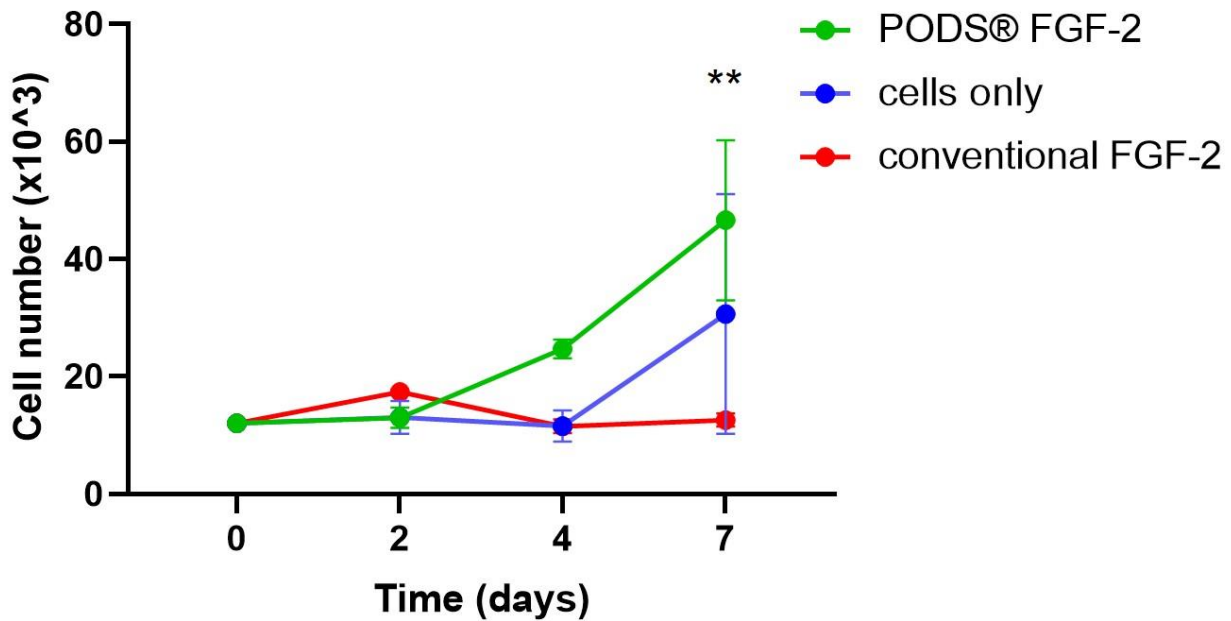


Figure 3. Quantification of NIH 3T3 mouse fibroblast cell numbers embedded in Gamma2 PeptiGel®, either alone, with supplemented conventional FGF-2, or with PODS® FGF-2, in short-term culture. Cells were embedded into PeptiGel® and cultured under conditions as indicated. Cell number was quantified using the PicoGreen® reagent at different time points. Statistical analysis was performed using 2-way ANOVA (**p<0.01, n≥2).

Conclusions

- Presence of PODS® growth factors in the PeptiGel® can enhance initial cell survival above the levels achieved using conventional soluble growth factors.
- Presence of PODS® proteins increases cell proliferation and activity above the levels achieved using conventional growth factors.
- The effect of PODS® growth factors can be achieved with a single application of PODS® when the experiment is set up without further intervention, whereas soluble growth factor requires frequent replenishment.

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

Growth Factors

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- Purification
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