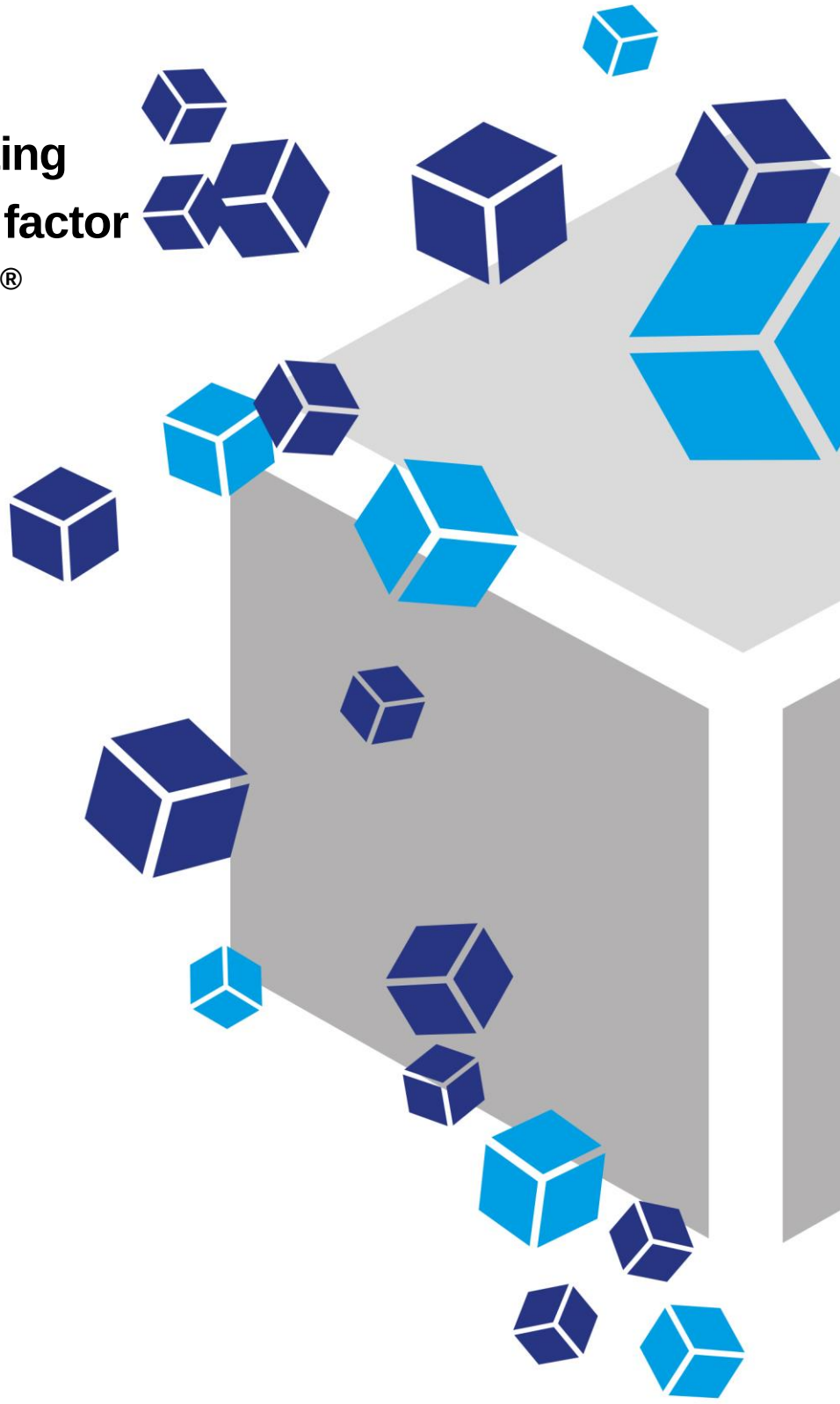
Application Note

Chemotaxis: Generating Neurotrophic growth factor Gradients with PODS®



PODS® Neurotrophin-generated gradients induce chemotactic alignment and differentiation of nerve cells

Data Courtesy of Prof. Hajime Mori, Kyoto Institute of Technology, Japan

Introduction to PODS®

The challenge for conventional growth factors

Many proteins, especially growth factors and cytokines, when used as a reagent, degrade quickly, rapidly losing their bioactivity. Additionally, they can also suffer from lot-to-lot product variation. This fragility and variability hampers research and significantly limits the therapeutic potential of proteins.

Protein Micro-depots

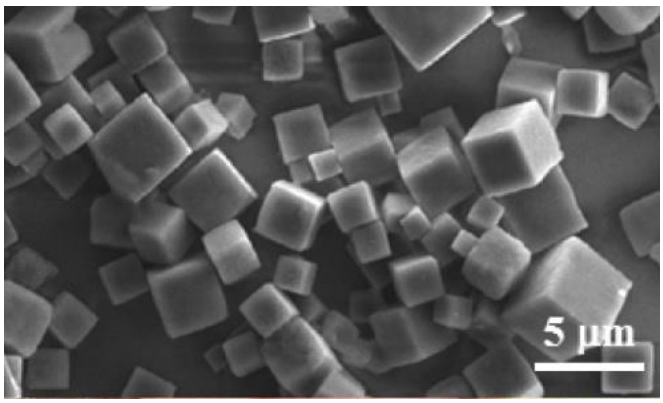
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

Introducing PODS®

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® typically release intact cargo protein over several weeks and 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:

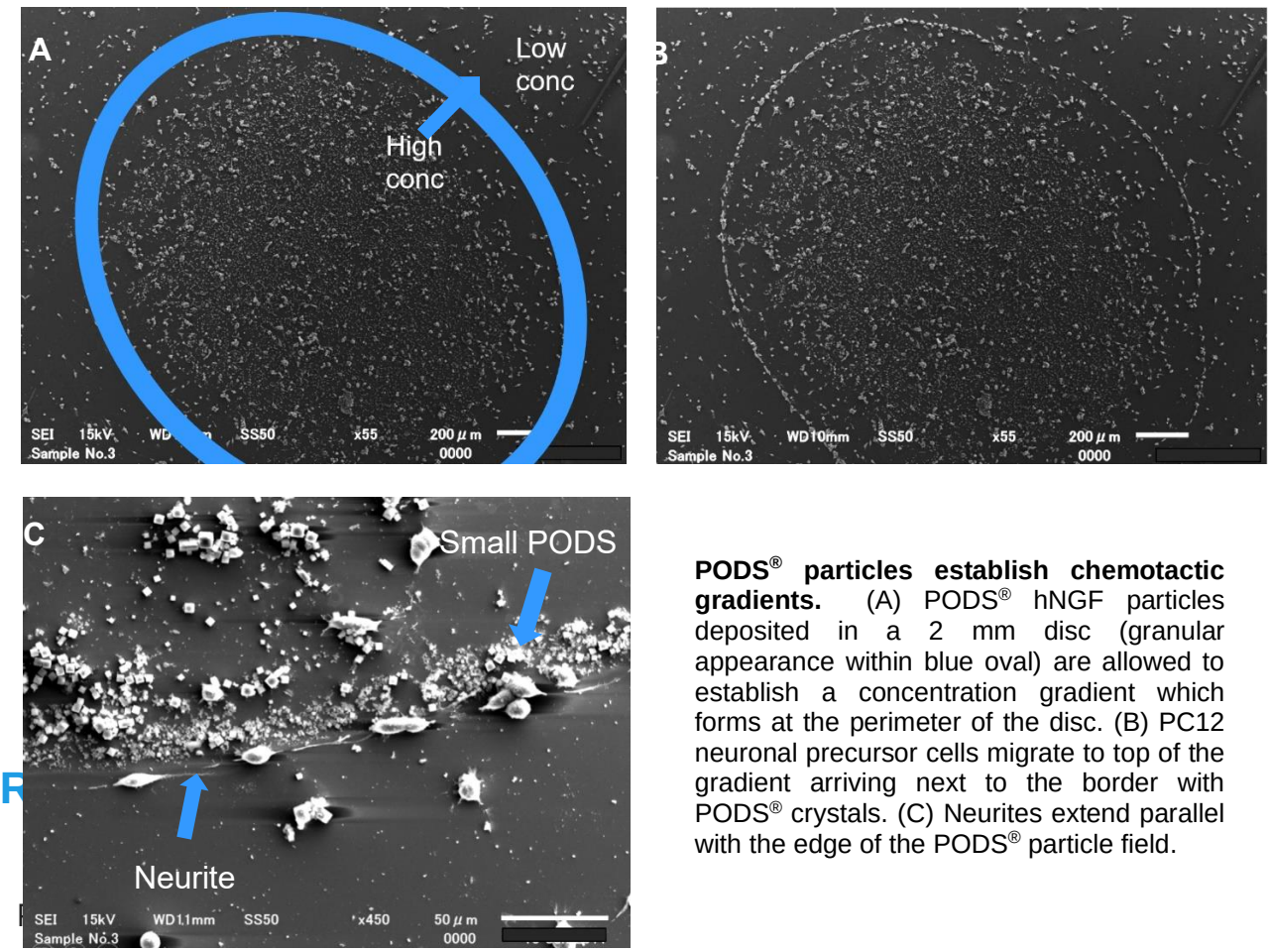
- Micropatterning
- Physiological, stable gradient formation
- Bioinks for 3D printing
- Microcarriers
- Functionalizing scaffolds
- Microfluidics (lab on a chip)
- Improved and simplified stem cell culture
- Therapeutic protein delivery

Overview:

The spatial and temporal availability of cytokines, and the microenvironments this creates, is critical to tissue development and homeostasis. Creating concentration gradients in vitro using conventional recombinant proteins is challenging as they do not provide a self-sustainable source. Here, PODS® particles containing human-derived nerve growth factor (hNGF) were used in a very simple experiment which created a concentration gradient simply using a standard petri dish. These results were achieved without the need for any devices or pumps.

Methods

PODS® hNGF particles (Cat PPH316) were pipetted onto the centre of a petri dish in a volume of 2µl of PBS and allowed to dry. This created a disc of PODS® particles in the centre of the dish with a diameter of approximately 2 mm. PC12 cells (derived from a pheochromocytoma of the rat adrenal medulla, that have an embryonic origin from the neural crest that has a mixture of neuroblastic cells and eosinophilic cells) were seeded onto the dish in DMEM medium supplemented with 10% horse serum. The plate was incubated static, without movement or agitation, for 96 hours. The plate was examined under scanning electron microscopy.



PODS® particles establish chemotactic gradients. (A) PODS® hNGF particles deposited in a 2 mm disc (granular appearance within blue oval) are allowed to establish a concentration gradient which forms at the perimeter of the disc. (B) PC12 neuronal precursor cells migrate to top of the gradient arriving next to the border with PODS® crystals. (C) Neurites extend parallel with the edge of the PODS® particle field.

Results

PODS® particle sustainably secrete their cargo protein. This creates gradients around each individual PODS® particle. By clustering the PODS® particles in a discreet area, a regional gradient is set up around the cluster. After 4 days, the PC12 cells in the vicinity of the PODS® particles had migrated to the edge of the particle field to create a ring surrounding the PODS® particle disc. Notably, the PC12 cells had extended synaptic neurites towards each other. It was also remarkable that the ring of PC12 cells was not branched.

Conclusions

- PODS® particles sustainably secrete hNGF to form a chemotactic gradient
- PC12 cells responded to the NGF gradient to form a defined ring structure around the PODS® particle field
- PODS® particles simplify the study of chemotactic responses without the need to pumps and devices.

Further reading

Matsuzaki Y, Maruta R, Takaki K, Kotani E, Kato Y, Yoshimura R, Endo Y, Whitty C, Pernstich C, Gandhi R, Jones M, Mori H. (2019). Sustained Neurotrophin Release from Protein Nano-particles Mediated by Matrix Metalloproteinases Induces the Alignment and Differentiation of Nerve Cells. **Biomolecules** 20;9(10)

For more information and a full list of our current PODS® growth factors, please visit our website www.cellgs.com.

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

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