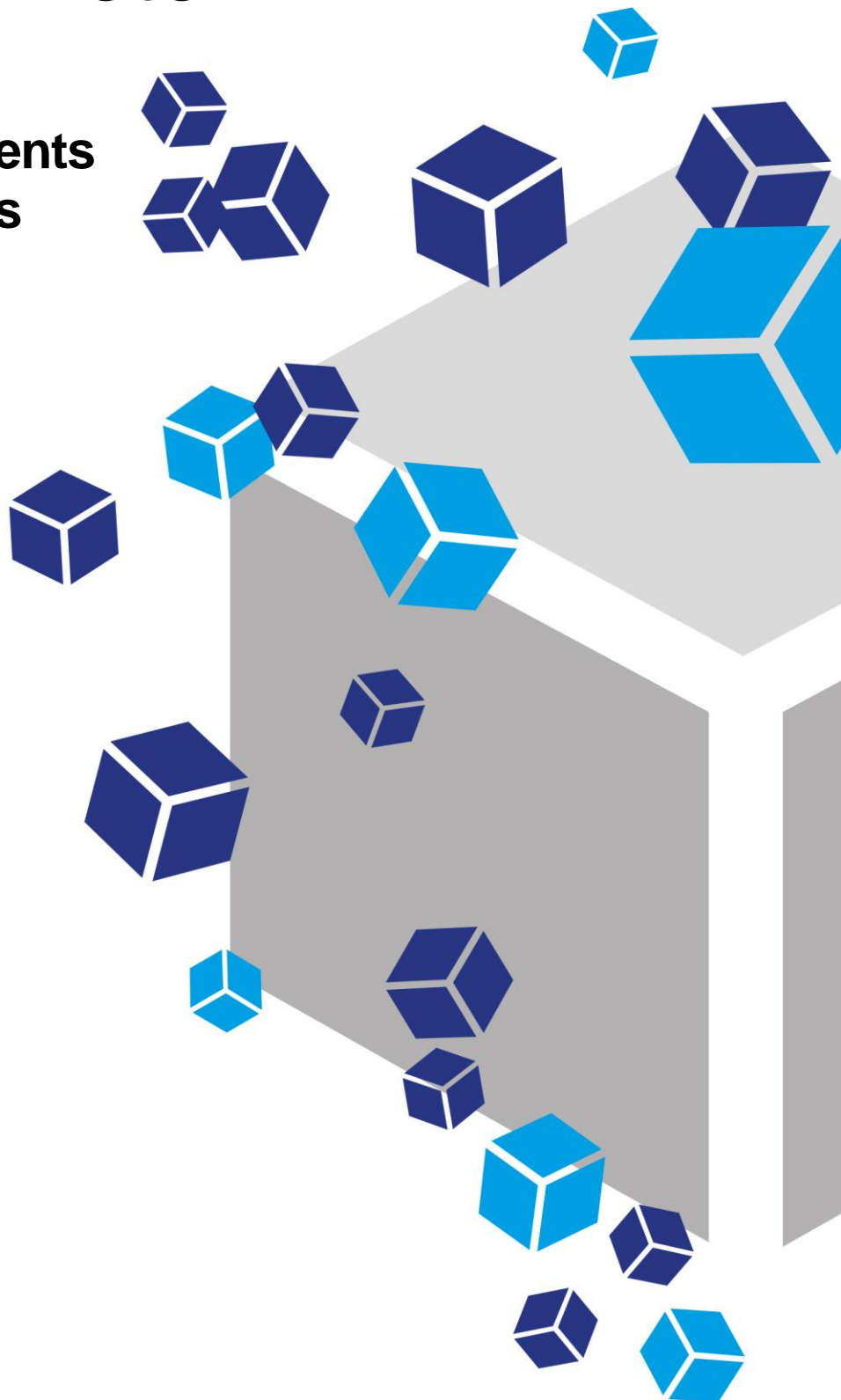


Application Note

**Generating
growth factor gradients
with PODS[®] crystals**



Achieving growth factor gradients utilizing PODS[®] BMP-2

Data Courtesy of Takaki Shima, HI-LEX Corporation
Takarazuka, Hyōgo, Japan (Issued March, 10th 2019)

Introduction to PODS[®]

The challenge with soluble growth factors

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

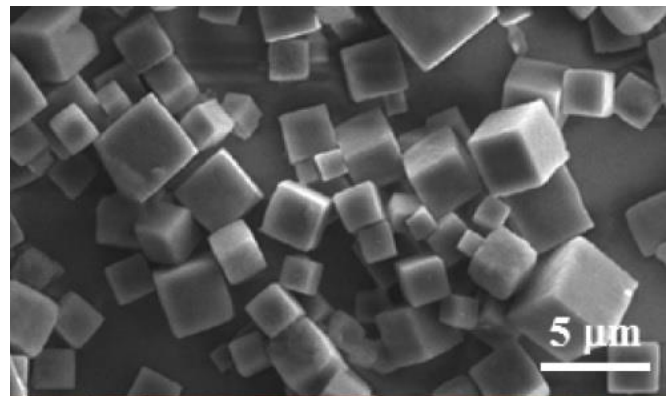
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control over the growth of cells.

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.

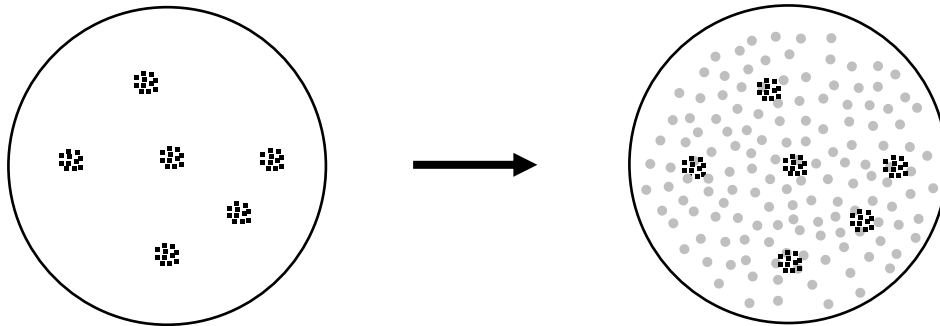


How can PODS[®] help my research?

PODS[®] are tough and will withstand physical and chemical stress, so you can handle them with ease. PODS[®] can be made to release intact cargo protein over days, weeks or even 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

Methods



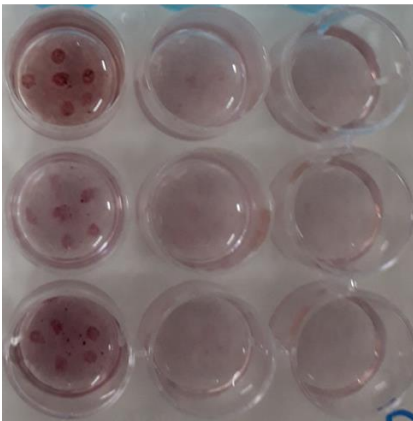
Culture method: PODS[®] BMP-2 and PODS[®] Empty crystals were spotted as six dense discs into wells of a 24-well plate and dried on (black squares). ATDC5 cells, a chondrogenic cell line, were then seeded across the entire well and cultured for 9 days. **NOTE:** a single application of PODS[®] crystals was used during the culture period.

Staining: On Day 9, cells were fixed in methanol. Subsequently, cells were either stained with Alizarin red for calcification, or stained with Alcian blue for extracellular matrix (ECM) production.

Results

A

PODS[®]
BMP-2 PODS[®]
Empty Cells
only

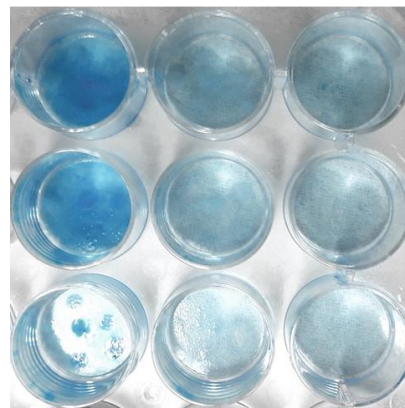


(A) Alizarin red staining of ATDC5 cells

ATDC5 cells stained with Alizarin red to indicate osteogenic differentiation (calcification). PODS[®] BMP-2 stimulates calcification (red spots, left column), whereas cells cultured in the presence of PODS[®] Empty crystals or cells alone did not show signs of osteogenic differentiation. Only the cells in close vicinity of the PODS[®] BMP-2 field stained red, demonstrating build-up of BMP-2 around the PODS[®] crystals.

B

PODS[®]
BMP-2 PODS[®]
Empty Cells
only



(B) Alcian blue staining of ATDC5 cells

ATDC5 cells stained with Alcian blue to indicate chondrogenic differentiation (glycosaminoglycan synthesis). Analogous to the Alizarin red staining, only PODS[®] BMP-2 crystals were able to stimulate ECM production in ATDC5 cells (blue spots, left column), whereas cells that were cultured in the presence of PODS[®] Empty crystals or cells alone did not show signs of differentiation towards cartilage formation.

Conclusions

- PODS[®] crystals adhere efficiently to plastic surfaces, ideal for localized placement on tissue culture dishes.
- PODS[®] achieve growth factor gradients easily when positioned in high densities in confined areas.
- Even for long culture periods, a single application of PODS[®] crystals is effective, significantly reducing both hands-on time and cost of materials.
- PODS[®] BMP-2 exhibits bioactivity.

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*

Growth Factors

- Recombinant
- Sustained Release

Exosomes

- Purification
- Detection
- Tracking
- NTA Service

Small Molecules

Cell Counting Reagent

Matrix Proteins

Cell Culture Media

- Pluripotent Stem Cells
- Photostable
- *In Vitro* Blastocyst Culture
- ETS-embryo Culture
- Custom Manufacturing Service

Gene Knock-Up System

Cytogenetics Analysis



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