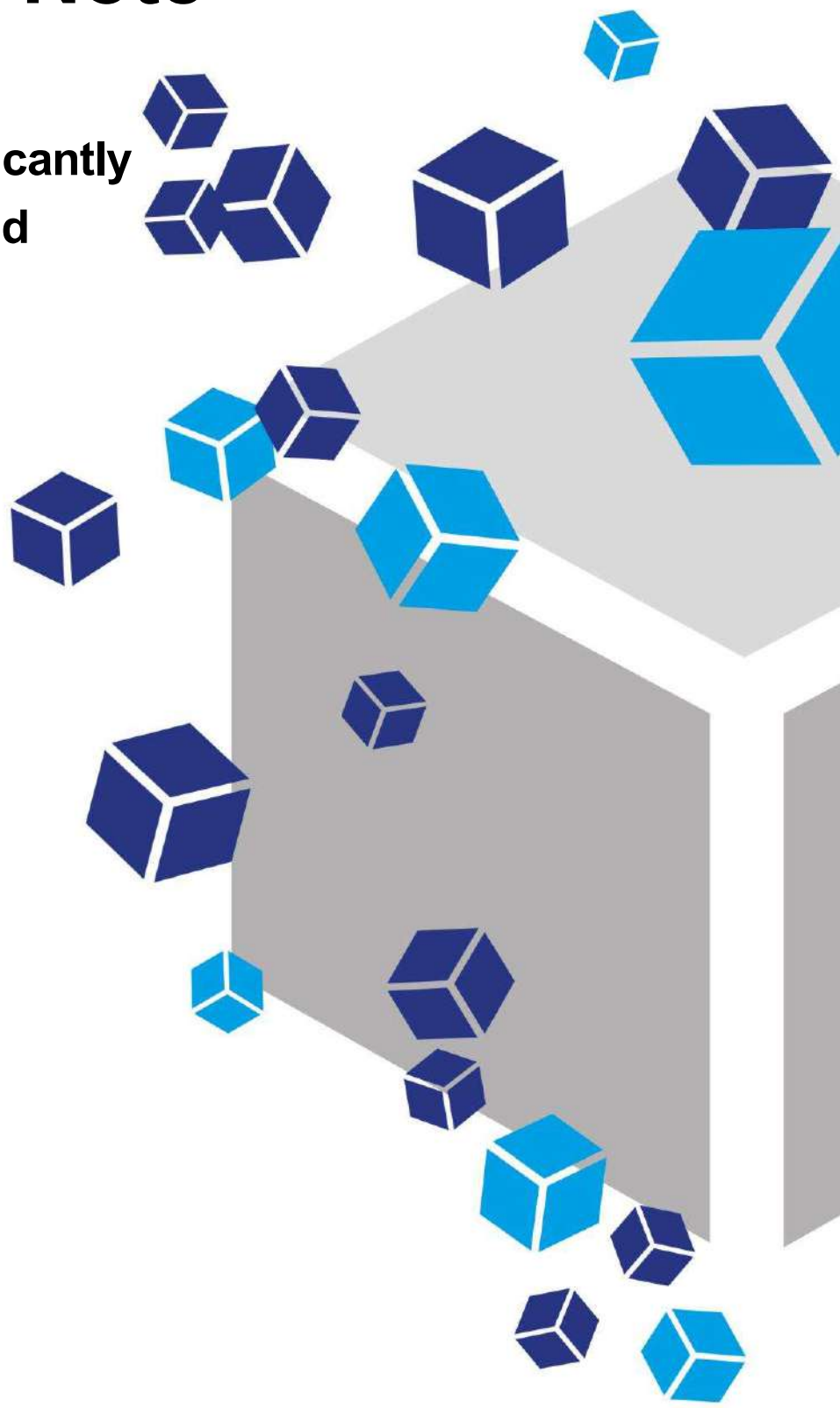
CELL guidance systems

Application Note

PODS® hBDNF significantly enhances survival and differentiation of implanted neurons



PODS® hBDNF significantly enhances survival and differentiation of implanted neurons

Data Courtesy of Dr Akihiro Matsuoka, Northwestern University, Chicago, IL, USA

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

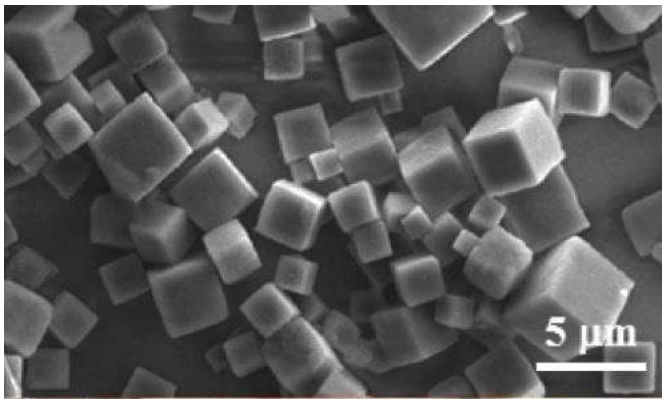
Development of a technology that can continuously replenish active protein from a local, microscopic store, has been a significant challenge, but one that could transform the fields of cell culture and medicine by allowing greater control and reproducibility of cell culture.

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:

Stem cell therapy is being developed to replace neurons lost through disease or injury. However, its potential is hindered by low survival rates and poor maturation of cells post transplantation. Here, we review the outstanding *in vivo* neuroprotective and maturation-promoting effects of PODS® human brain neurotrophic growth factor (hBDNF). The neuroprotective effects of PODS® hBDNF *in vitro* are detailed in a companion Application Note.

The effects of neurotrophic growth factors on auditory neurons has been widely studied. During sensorineural hearing loss, destruction of hair cells in the cochlea occurs. Since these cells are a major source of neurotrophic factors, this causes retrograde trans-synaptic degeneration resulting in the death of afferent auditory neurons. hBDNF has been shown to promote survival and neuronal differentiation of spiral ganglion neurons both *in vitro.* However*, in vivo* effects of conventional hBDNF are limited by its short half-life.

The harsh, nutrient-deprived environment of the cochlea is a major challenge for survival of implanted cells. To promote the *in vivo* survival and neuronal differentiation of transplanted human Embryonic Stem Cell (hESC)-derived Otic Neuronal Progenitors (ONPs), PODS® hBDNF crystals were utilized as a long-term neurotrophic growth factor delivery system. Sustained hBDNF release from a single dose of PODS® hBDNF enhanced survival and neuronal differentiation of ONP spheroids towards otic neuronal lineages. Furthermore, PODS® hBDNF efficiently promoted neurite extension towards the bony wall of the cochlea during a 90-day post-transplantation period.

Methods

In vivo transplantation of hESC-derived ONPs into the inner ear

For a detailed protocol, please refer to the original manuscript[1]. Briefly, hESC-derived late-stage ONP spheroids were generated and either i) dissociated into single cells; or ii) left as spheroids. Both groups were prepared for transplantation into the inner ear of mice. For each 2µl transplant, either $0.25x10^6$ hESC-derived ONPs or 5 hESC-derived ONP spheroids (each containing $5x10^4$ ONP cells) was used. Prior to transplant, the cells or spheroids were mixed with either (i) neuronal induction media (NIM) alone; (ii) 1% GrowDex®-T hydrogel (UPM Biomedicals, catalogue number: 200103002); or (iii) 1% GrowDex®-T functionalized with $8x10^5$ PODS® hBDNF crystals (catalogue number: PPH1) (Figure 1). Transplants were performed at postnatal day 40 (P40) into the left cochlea of deafened Diphtheria Toxin Receptor-positive (DTR^+) mice. The right cochlea was used as a control.

Following transplantation, the mice were allowed to recover and kept for 90 days, allowing engraftment and neuronal differentiation of the transplanted cells within the inner ear. No immunosuppressant medication was administered. After completion of the post-implantation survival period (P130), each animal was euthanized, the cochlea dissected and histological analysis performed.

Results

PODS® hBDNF treatment supports the long-term *in vivo* survival and neuronal differentiation of hESC-derived ONP spheroids into the cochlea of DTR^+ mice.

90 days post-implantation, ONP spheroids transplanted with GrowDex®-T or PODS® hBDNF/GrowDex®-T engrafted most efficiently and maintained aggregated forms in the inner ear (Figure 2). Identification of hESC-derived ONP cells that survived post-implantation was based on the pan-neuronal

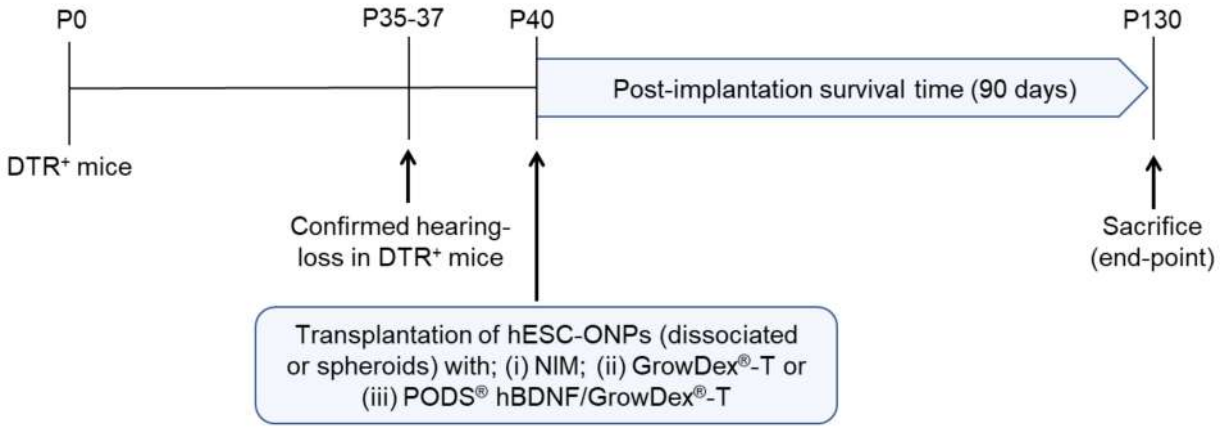


Figure 1. In vivo transplantation of hESC-derived ONPs with PODS® hBDNF/GrowDex®-T into the DTR⁺ mouse cochlea. A schematic diagram of experimental design for *in vivo* hESC-derived ONP transplantation with NIM (only); GrowDex®-T or PODS® hBDNF/GrowDex®-T. Figure adapted from (1).

marker β-III tubulin positive staining (green) and anti-human nuclear antibody (AHNA) positive staining (red), to distinguish implanted cells from residual endogenous mouse cells. ONP spheroids primarily lodged within the scala tympani. In contrast to cell transplanted with GrowDex®-T alone (Figure 2a), cells transplanted with PODS® hBDNF/GrowDex®-T exhibited neurites greatly extended toward the modiolus (Figure 2b; white arrows).

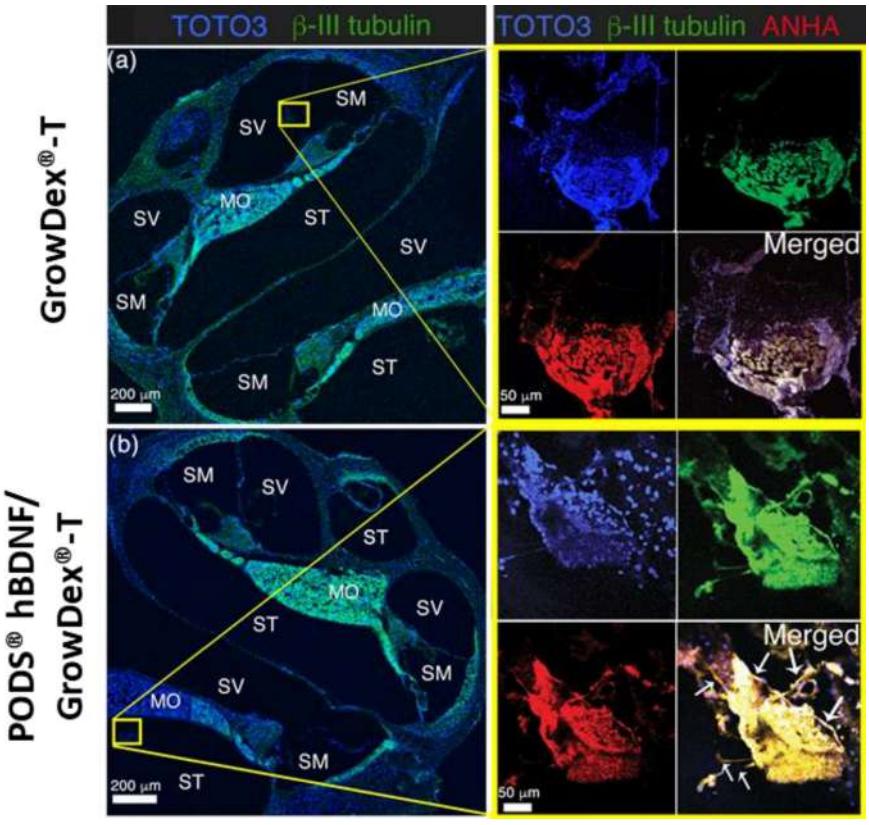


Figure 2. PODS® hBDNF treatment supports the long-term *in vivo* survival and neuronal differentiation of hESC-derived ONP cells transplanted in DTR⁺ mice cochlea. Immunohistochemistry of transplanted hESC-derived ONP spheroids in the DTR⁺ mouse cochlea with (a) GrowDex®-T (only) or (b) PODS® hBDNF/GrowDex®-T. Left panels: Low magnification (10X) microphotographs of the DTR⁺ mouse cochlea. Each yellow square indicates the anatomical location of a transplanted hESC-derived ONP spheroid. Right panels: High magnification (40X) microphotographs of transplanted hESC-derived ONP spheroids stained with nuclear marker TOTO3 (blue), β-III tubulin (green), and AHNA (red). Small white arrow: neurites. SM: scala media, SV: scala vestibuli, ST: scala tympani, MT: the middle turn of the cochlea, and MO: modiolus. Figure adapted from (1).

Quantification of AHNA-positive staining indicates that dissociated cells did not survive transplantation as efficiently as those in spheroid form, irrespective of the engraftment location in the cochlea (Figure 3a). Further analysis confirmed that engraftment was significantly better with GrowDex®-T than NIM only and was further enhanced when functionalizing the hydrogel with PODS® hBDNF (Figure 3b). The effect of the sustained-release of neuroprotective hBDNF from PODS® was most pronounced with dissociated ONP cells. The overall survival rate of transplanted spheroids with PODS® hBDNF/GrowDex®-T after 90 days was approximately 0.1%. Quantitative analysis further demonstrated that PODS® hBDNF markedly enhanced neurite outgrowth of engrafted ONP spheroids (Figure 3c).

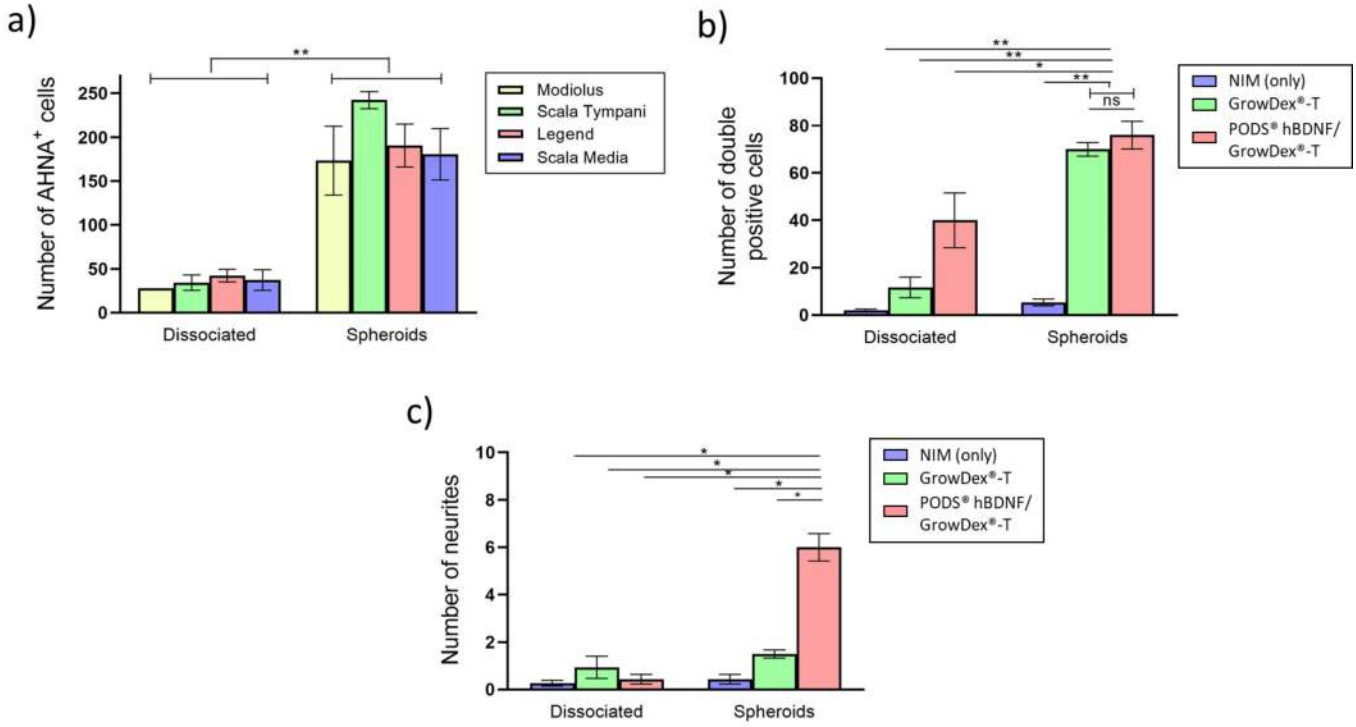


Figure 3. PODS® hBDNF treatment supports the long-term *in vivo* survival and neuronal differentiation of hESC-derived ONP cells transplanted in DTR$^+$ mice cochlea. a) Quantification of the number of the ANHA-positive cells in dissociated ONPs transplantation *vs* ONP spheroids transplantation in four anatomical subdivisions of the cochlea. b) Quantification of the number of double positive cells (ANHA$^+$ and βIII-Tubulin$^+$) in dissociated ONPs transplantation *vs* ONP spheroid transplantation with three different conditions: NIM only; GrowDex®-T; PODS® hBDNF/GrowDex®-T. c) Quantification of the number of neurites in dissociated ONPs transplantation *vs*. ONP spheroid transplantation. $*p < 0.05$, $**p < 0.01$, N.S.: not significant by one-way ANOVA with Tukey's *post-hoc* test. Figure adapted from (1).

Taken together, this data demonstrates that *in vivo* transplanted hESC-derived ONP spheroids embedded in PODS® hBDNF/GrowDex®-T hydrogel greatly enhances survival and further neuronal differentiation into otic neuronal lineages, as demonstrated by extended neurites towards the bony wall of the cochlea.

Conclusions

- PODS® hBDNF enhances the survival rate of hESC-derived ONP spheroids *in vivo*, 90 days post-transplantation in the inner ear.
- PODS® hBDNF significantly enhances neurite extension and axonal branching of engrafted hESC-derived ONP spheroids *in vivo.*
- PODS® crystals can be readily incorporated into nanofibrillar cellulose hydrogels to functionalize these by delivering bioactive growth factors in a sustained manner.
- A single dose of PODS® crystals can deliver growth factors localized *in vivo* without the use of immunosuppressant medication. PODS® may therefore be ideal for therapeutic use within the inner ear.

Reference

1) Chang HT, Heuer RA, Oleksijew AM, Coots KS, Roque CB, Nella KT, McGuire TL, Matsuoka AJ. An engineered three-dimensional stem cell niche in the inner ear by applying a nanofibrillar cellulose hydrogel with a sustained-release neurotrophic factor delivery system. Acta Biomater. 2020 May;108:111-127.

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

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- ExoLISA™ - ELISA-like detection
- Instant Exosomes™ - purified and characterized
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- Freeze drying service

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