Engineering a Three-Dimensional Stem Cell Niche in the Inner Ear by Applying a Nanofibrillar Cellulose Hydrogel with a Sustained-Release Neurotrophic Factor Delivery System

Hsiang-Tsun Chang[1], Rachel A. Heuer[1], Andrew M. Oleksijew[1], Kyle S. Coots[1], Christian B. Roque[1], Kevin T. Nella[1], Tammy L. McGuire[2], and Akihiro J. Matsuoka[1,3,4]

[1]Department of Otolaryngology-Head and Neck Surgery, [2]Department of Neurology, [3]Department of Communication Sciences and Disorders, and [4]Knowles Hearing Center, Northwestern University

Background

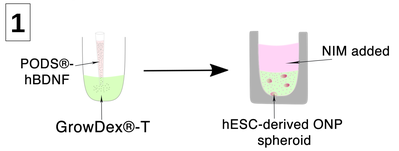
The application of human embryonic stem cells (hESCs) in stem cell replacement therapies in the inner ear is hindered by low cell survival post-transplant. Here, we aim to enhance the in vitro and in vivo survival rate and neuronal differentiation of otic neuronal progenitors (ONPs) by generating an artificial stem cell niche consisting of three-dimensional (3D) hESC-derived ONP spheroids, a nanofibrillar cellulose hydrogel, and a sustained-release brain derivative neurotrophic factor (BDNF) delivery system.

Research Objectives

- Create hESC-derived ONP spheroids for transplant to the inner ear
- Create an in vitro spheroid niche using a sustained nutrient supply and a nanofibrillar cellulose hydrogel as an extracellular matrix (ECM)
- Analyze neuronal characteristics of the spheroids in vitro to explore the effects of the niche components
- Examine transplanted samples for spheroid survival, engraftment, and neurite extension

Methods

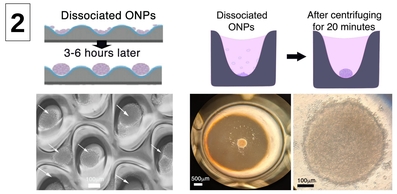
1. Generation of ONP spheroids: hESC differentiation has been previously described [1]. At the ONP stage, cells were seeded to 3D spheroids using a 96-well Clear Round Bottom Ultra-Low Attachment Microplate® (Corning Life Science) or an EZsphere® culture plate (Nacalai).
2. Creation of in vitro stem cell niche: ONP spheroids were cultured with neural induction medium (NIM), Growdex®-T (UPM-Kymmene Corporation) diluted to 0.375% (w/v) as ECM, and PODS®-hBDNF (Cell Guidance System), crystalline structures designed to release BDNF at a steady rate into medium. See diagram at right.
3. In vitro analysis: BDNF secretion by PODS® was measured using an enzyme-linked immunosorbent assay (ELISA). Neurite growth of spheroids was analyzed with a neurite arborization area assay.
4. In vivo transplantation: ONP spheroids or dissociated cells were transplanted into the left cochleae of deafened mice with Growdex®-T and PODS®-hBDNF. After sacrifice and sectioning, slides were stained using an anti-human nuclear antibody (AHNA) to track ONPs and calcofluor to track the Growdex®-T hydrogel.



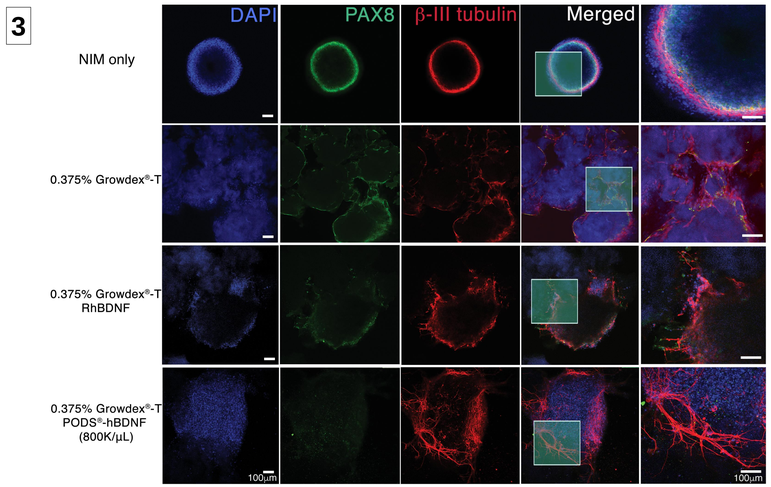
(1) Schematic of culture of hESC-derived ONP spheroids in a Growdex®-T ECM with PODS®-hBDNF and NIM

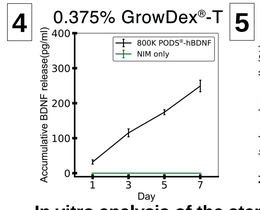
1. Matsuoka AJ, Morrissey ZD, Zhang C, Homma K, Belmadani A, Miller CA, et al. Directed differentiation of human embryonic stem cells toward placode-derived spiral ganglion-like sensory neurons. Stem Cells Transl Med. 2017 Mar;6(3):923–36.

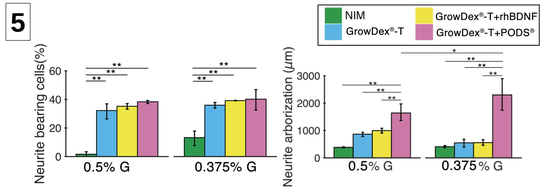
Results



Generation of ONP spheroids. (2) Top: schematic of spheroid formation by EZsphere® plate (left) or 96-well ultra-low attachment microplate (right). Bottom: phase contrast photomicrographs of individual spheroids (left: EZsphere®, center: 96-well, right: 96-well close-up).





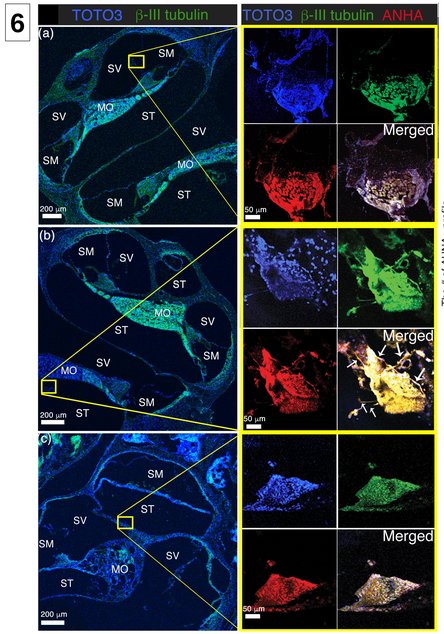


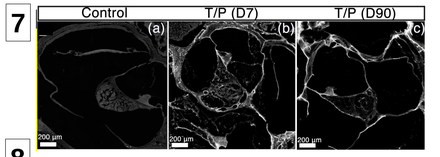
In vitro analysis of the stem cell niche. (3) Immunocytochemistry of ONP spheroids cultured for seven days in NIM only, with Growdex®-T, with Growdex®-T and 20ng/mL recombinant human BDNF (RhBDNF), or with Growdex®-T and 800,000 PODS®-hBDNF. βIII tubulin staining (red) shows neurites. (4) Accumulative BDNF release profile of 800,000 PODS®-hBDNF in 0.375% GrowDex®-T over seven days. (5) Quantification of neurite bearing cells and neurite arborization area for each culture condition. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by one-way ANOVA with Tukey's post-hoc test.

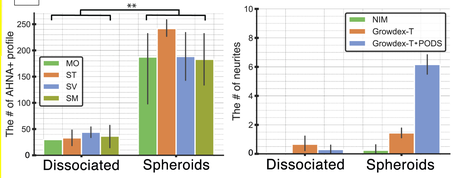
Disclosure

There are no financial conflicts of interest to disclose. Any kind of financial support (funding, grants, sponsorship) received has been acknowledged (see the right column)

Results (Continued)







Post-transplant analysis of ONP spheroids. (6) Immunohistochemistry of transplanted hESC-derived ONP spheroids in mice cochleae, low-power (left) and high power (right) magnification. ANHA: anti-nuclear human antibody, SV: scala vestibuli, SM: scala media, ST: scala tympani, MO: modiolus. Arrows: neurites. (7) Calcofluor staining of cellulose in Growdex®-T. a: non-transplanted control, b: specimen sacrificed 7 days after transplant, c: specimen sacrificed 90 days after transplant. (8) Number of AHNA-positive cells (left) and neurites (right) visualized after transplant of either dissociated cells or spheroids (250,000 cells or five spheroids of 50,000 cells each). ** $p < 0.01$ by one-way ANOVA with Tukey's post hoc test.

Conclusions

Our data demonstrate the importance of an appropriate stem cell niche for transplanted cell survival and the ability of the Growdex®-T/PODS®-hBDNF system to fill this role for hESC-derived ONP spheroids. In vitro, PODS® released BDNF at a steady rate into the medium, and PODS® and Growdex®-T supported an increase in neurite-bearing cells and in neurite extension. In vivo, our spheroids were shown to support greater cell survival after transplant than dissociated cells, as well as increased neurite extension. Our protocol represents a significant step towards the integration of transplanted hESC-derived ONPs into the inner ear, a necessary process to expand stem cell replacement therapies in the inner ear.

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