GrowDex®-T

Application Note 5

UPMBIOFORE BEYOND FOSSILS

Neuronal Differentiation of Human Embryonic Stem Cell Derived Otic Neural Progenitors Using a Nanofibrillar Cellulose and Hydrogel Scaffold and Polyhedrin Delivery System

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INTRODUCTION

Stem cell therapy is a promising method to alleviate sensorineural hearing loss, but its potential is currently hindered by low cell survival post transplantation (1-4). The harsh, nutrient deprived environment of the cochlea is thought to be a leading factor in poor cell survival (5,6). To alleviate this problem, we have proposed to use GrowDex®-T as an artificial extracellular matrix that is capable of both providing scaffolding for cells to attach and as a way to keep slow releasing brain derived neurotrophic factor (BDNF) PODSTM -crystals in place.

MATERIALS

- GrowDex-T 1% (Cat No. 200 103 005, UPM)
- EZSPHERE™ (Nacalai)
- BrainPhys™ Neuronal Medium (Cat No. 05790, Stem Cell Technologies)
- BDNF-PODS™ (Cat No. PPH1-250, Cell Guidance Systems)
- DMEM/F-12 supplemented with 1% N2 supplement, 2% B27 supplement, 2 mM glutamine, 100 µM beta-mercaptoethanol (all reagents were obtained from Life Technologies)

UPMBIOMEDICALS

METHODS

Human embryonic stem cell (hESC) derived late-stage otic neural progenitors (ONPs), as previously described and characterized (7), were single dissociated from a traditional monolayer plate and transferred to a micro-fabricated three dimensional cell culturing device (EZSPHERE, Nacalai) that consists of uniformly sized microwells coated with a low-binding affinity polymer (2-methacryloylxyethyl phosphorycholine). Cells were cultured in an ONP maintenance media (ONPMM) (7) at 37°C, 5% CO2 for two days and allowed to form spheroids with diameters of roughly 250 µm.

1. GrowDex-T stock was diluted to 0.25% (v/v) with PBS (-/-).
2. 100 µl of 0.25% GrowDex-T was plated into a 48-well plate.
3. Spheroids were transferred into the GrowDex-T using a P200 micropipette tip and 500 µl of BrainPhys was gently added on top of the mixture.
4. 75,000 BDNF-PODS (equal to 10 ng/ml) were added to the GrowDex-T.
5. Spheroids were cultured for 7 days at 37°C, 5% CO2 with no media changes.
6. Following 7 days, the media was removed and the cells and GrowDex-T were fixed with 4% PFA and fluorescently stained for Peripherin, VGLUT2, and DAPI.

RESULTS

Immunocytochemical (ICC) analysis shows that the hESC-derived ONP spheroids stained positive for DAPI, Peripherin (peripheral neuron marker) and VGLUT2 (glutamate transporter marker) after one week in differentiation media. Z-stack (n=18) photomicrograph (Fig. 1) obtained using a Nikon A1(C) confocal microscope. Image shown from the ninth plane. Note that the 3D culture condition produces multiple image planes; differing apparent fluorescence intensities are caused by out-of-focus cells within spheres.

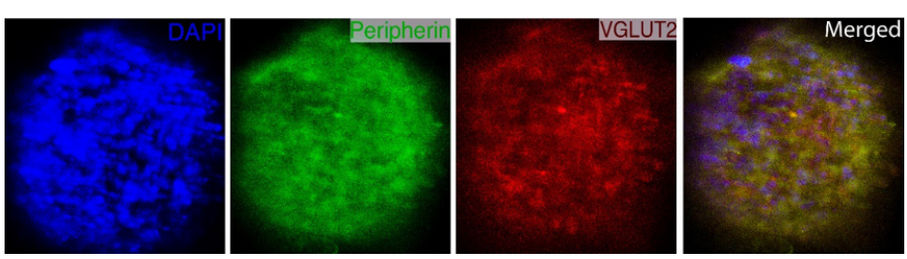

Figure 1. A Photomicrograph of ICC image on an immature glutamatergic neuronal spheroid stained with DAPI, Peripherin, and VGLUT2. Scale bar: 100 µm.

CONCLUSIONS

Our results indicate that 0.25% GrowDex-T allows for robust differentiation of LONPs into immature glutamatergic peripheral neurons when supplemented with 75,000 BDNF-PODS. In conjunction with slow-releasing BDNF with PODS crystals, GrowDex-T can be a suitable artificial extracellular matrix that is potentially capable of facilitating survival and neurite growth of hESC-derived neuronal progenitors in the future.

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