Biopreservation of Living Tissue Engineered Neural Networks Under Hypothermic Conditions for Efficient Storage and Transport

Dimple Chouhan[a,b], Wisberty Gordián-Vélez[a,b,c], Kevin D. Browne[a,b], Bella Chau[a,b], Robert B. Shultz[a,b], D. Kacy Cullen[a,b,c]

[a]Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
[b]Center for Neurotrauma, Neurodegeneration & Restoration, CMC Veterans Affairs Medical Center, Philadelphia, PA
[c]Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA

Statement of Purpose: Living tissue engineered constructs have made immense progress in recent years, leading to increased efforts to translate these products from bench to bedside to treat and repair myriad conditions. The commercial viability of tissue engineered constructs not only requires efficacy in their intended clinical application but also efficient transportation and storage conditions that preserve their beneficial properties. Herein, we have developed a strategy to biopreserve tissue engineered nigrostriatal pathways (TE-NSPs), an engineered construct developed for the treatment of Parkinson's disease (PD). These constructs have the potential to reconstruct the brain pathway that is lost in PD and, thus, restore the connectivity and dopamine signalling in the neural circuitry needed for proper motor function. These living neural implants are fabricated using dopaminergic neurons seeded in a hydrogel encasement that project long axons (e.g., centimeter-scale) to mimic the structure and function of the axon pathway between the substantia nigra and the striatum of the brain. In the present study, we investigated the temporary storage conditions under hypothermic (4-8 °C) conditions to meet the transportation requirements required to realize these TE-NSPs as a clinical product.

Methods: The TE-NSPs were fabricated using hydrogel micro-columns following previous protocols [1,2]. Briefly, photo-crosslinked methacrylated hyaluronic acid (MeHA) (3 % w/v) was used to make a micro-columnar hydrogel encasement that also featured a lumen with rat tail collagen type I (1 mg/mL) and mouse laminin (1 mg/mL). The micro-columns were seeded with human iPSC-derived dopaminergic neurons (iCell DopaNeurons from Fujifilm Cellular Dynamics). The TE-NSPs fabricated here had a 556 µm outer diameter (OD), a 300 µm inner diameter (ID) and total length of 6 mm. Once the axons achieved full length (>5mm) in the micro-columns by 18 days *in vitro* (DIV), they were hibernated under hypothermic conditions (4-8 °C) using a specialized hibernating media for 48 h. The hibernated TE-NSPs were then returned to 37 °C for 7 days to examine their viability under physiological conditions. To further test their viability, they were also subjected to conditions mimicking physiological O_2 levels presenting 5 % O_2 levels (physoxia) at 37 °C post-hibernation. Viability was assessed by using a live/dead assay [4 µM calcein acetoxymethyl (Calcein AM), 2 µM ethidium homodimer-1 (EthD-1)] and imaging constructs with confocal microscopy.

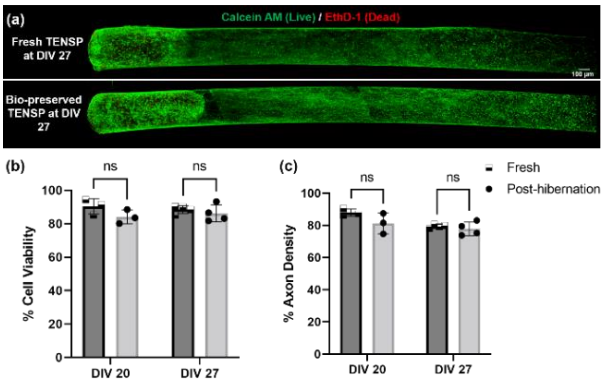

Figure 1: Representative confocal microscopic images of TE-NSPs depicts (a) comparable viability between fresh and hibernated constructs upon live/dead assay; scale bar: 100 µm. Quantitative analysis confirmed (b) equivalent cell viability between fresh and hibernated cultures at 20 DIV (pre-hibernation) and 27 DIV (post-hibernation) time points, and (c) comparable axon density, which suggested no effect on axonal health post-hibernation.

Results: We found that hibernated TE-NSPs had similar viability as their age-matched fresh counterpart cultures (Fig 1). The viability of both fresh and hibernated constructs ranged between 80-95 %, without any effect on axonal density. The hibernated TE-NSPs demonstrated similar viability before and after their hibernation. Moreover, the TE-NSPs cultured under physoxia conditions were equally viable, which provided clues to their survival in the brain environment post-transplant.

Conclusions: Short-term preservation of implantable neural constructs, intended to treat neurodegenerative diseases, was examined to evaluate their transportation feasibility. Our finding suggests that the biopreservation protocol can bridge the spatiotemporal gap between the biomanufacturing source and the implant destination for tissue-based clinical products, thereby enabling easy transportation and distribution. In the future, we will examine long-term preservation to assess their storage life and functionality of biopreserved engineered tissues. These studies have widespread applicability and might allow longer shelf-life and effective shipping for tissue engineered medical products.

References: (1) Struzyna LA. J Tissue Eng Regen Med. 2018; 12: 1702-1716. (2) Gordian-Velez W. Brain Res. Bull. 2021; 175: 168-185.