Fabrication and Characterization of Hybrid Biomaterial Nerve Conduits for Neural Differentiation of Induced Pluripotent Stem Cells

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Introduction:

Neural tissue engineering approaches employ biomaterial scaffolds to mimic the microenvironment present in healthy tissue to direct stem cell differentiation into neural phenotypes [1]. We have recently reported the conditions necessary for preparation of three dimensional (3D) fibrin scaffolds for culturing induced pluripotent stem cells (iPSCs) with the goal of engineering neural tissue [2]. Our current objective is to combine 3D fibrin-based scaffolds and poly (ε-caprolactone) (PCL) electrospun fibers to produce biodegradable and biocompatible nerve conduits containing neural progenitors derived from iPSCs. In particular, we have made novel multifunctional hybrid natural-synthetic nerve conduits constructed of fibrin and retinoic acid (RA)-encapsulated nanofibers supported mechanically by PCL microfibers. Overall, iPSC-derived neural progenitors exhibited excellent viability and were able to migrate inside of these 3D engineered conduits.

Materials and Methods:

The process of electrospinning enables production of PCL nanofiber and microfiber scaffolds with a range of properties that can influence cell behavior. Retinoic acid, a small molecule that promotes stem cell differentiation into neural phenotypes, was successfully encapsulated inside the PCL nanofibers. Natural fibrin scaffolds were chosen since they could support iPSCs growth and proliferation. A fibrinogen solution was polymerized by the addition of thrombin and $CaCl_2$ into a fibrin gel "plug" at the base of each conduit. Whole or dissociated murine iPSCs EBs were added to a fibrinogen solution which, at the onset of polymerization, was injected by pipette into the lumen of each conduit. The fibrin gels were allowed to polymerize for 1 hour, after which conduits (Figure 1A and B.) were placed horizontally in each well of the 6-well plate, covered with 4 mL of cell media and incubated at 37 C and 5% CO_2. The viability of EBs seeded inside the fibrin scaffolds was

analyzed qualitatively after 10 days using a LIVE/DEAD® Viability/Cytotoxicity Kit (Invitrogen) (Figure 1C).

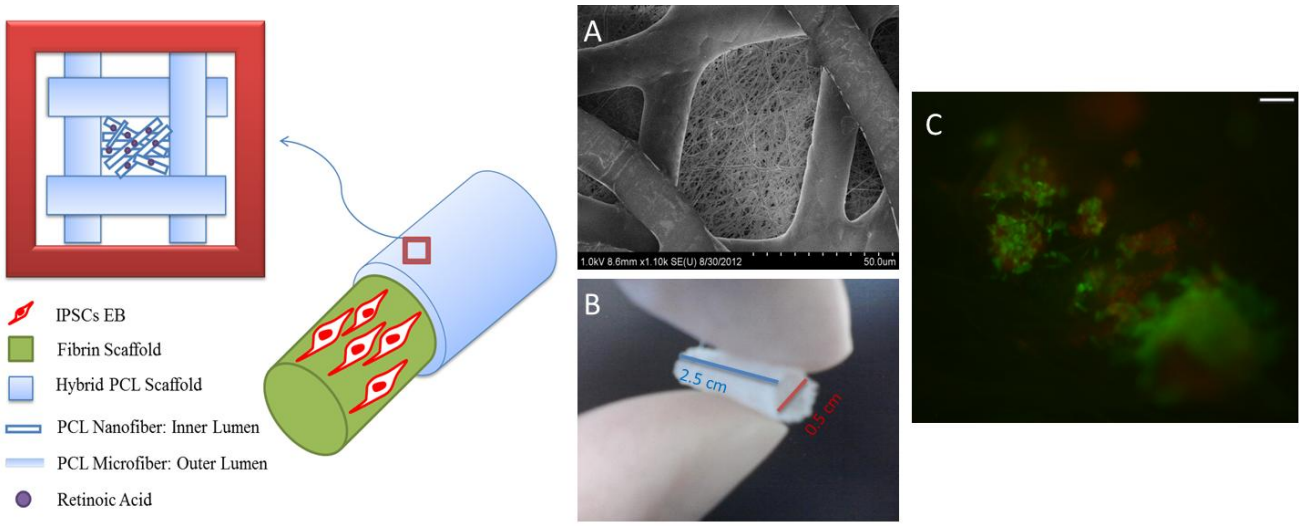

Figure 1. A schematic of multifunctional hybrid biomaterial nerve conduit. (A) A scanning electron microscopy (SEM) image of microstructure and nanostructure. (B) Image showing macrostructure. (C) Image from a LIVE/DEAD® cell viability assay on iPSC-derived EBs seeded inside the nerve conduit. Scale bar represents 100μm.

Results:

These novel hybrid biomaterial conduits could successfully support the culture of iPSC-derived neural progenitors as shown in Figure 1C. These cells were able to proliferate and migrate inside of this 3D microenvironment.

Discussion:

The ability of iPSC-derived neural progenitors to attach, survive and proliferate inside the nerve conduits was evaluated. This work showed that stem cell culture was feasible inside of these hybrid biomaterial nerve conduits. Our promising fabrication technique provided an excellent degree of reproducibility in controlling the fiber diameter, topography and mechanical properties. Moreover, controlled release of RA from PCL nanofibers was observed over 1 month. As future work, we will study the use of aligned encapsulated nanofibers and optimize these nerve conduits with the aim of enhancing neuronal differentiation of iPSCs through the controlled release of retinoic acid and also neural growth factors.

References:

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[2] Kolehmainen, K. and Willerth, S.M. Journal of Visualized Experiments: Bioengineering. 2012 Mar 2;(61):e3641.