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Using fibrous biomaterials to understand the role of the microenvironment during stem cell differentiation

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Introduction

The incorporation of biomaterials and three-dimensional (3D) structures to culture systems has changed how we approach basic problems *in vitro* by recreating more physiological relevant cellular models. Significant progress has been made in the development of biomaterials and their surfaces. We recapitulate different physical elements of the microenvironment using a synthetic fibrous platform to determine how the microenvironment contributes to cellular adhesion and biocompatibility of pluripotent stem cells throughout the entirety of the microenvironment, not just the surface. We define biocompatibility as sustaining an environment to foster cellular proliferation as well as maintain cellular function. In the case of pluripotent stem cells, we develop biomaterials to encourage cellular adhesion throughout the 3D structure as well as both maintain pluripotency and exhibit differentiation potential.

Materials and Methods

We have developed several different fibrous structures to serve as synthetic microenvironments to use for tissue engineering applications, specifically to evaluate the role of the microenvironment during stem cell differentiation.

Collagen yarns

- Bovine collagen fibers (provided by Kaneka North America)
- Ringspun into yarns with fineness Ne 20/1

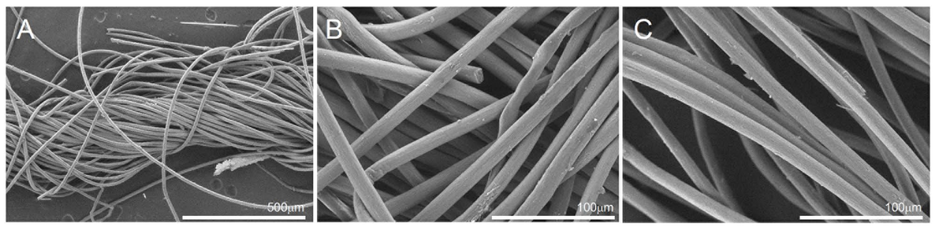

Figure 1: Collagen ringspun yarns *Scanning electron micrographs of collagen yarns.*

Electroconductive electrospun scaffolds

- Polycaprolactone (PCL) and gelatin were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol and extruded at a flow rate of 3mL/hr. 14kV was applied to electrospin onto copper shim collector.
- "Sandwich" scaffolds were made by electrospinning PCL-gelatin, carbon nanotube (CNT) arrays were manually stretched over the fibers and then another layer of PCL-gelatin was electrospun on top.
- "Dual deposition" (DD) scaffolds were created by simultaneously electrospinning PCL-gelatin under the same conditions as described while winding CNT arrays on to the same rotating mandrel.

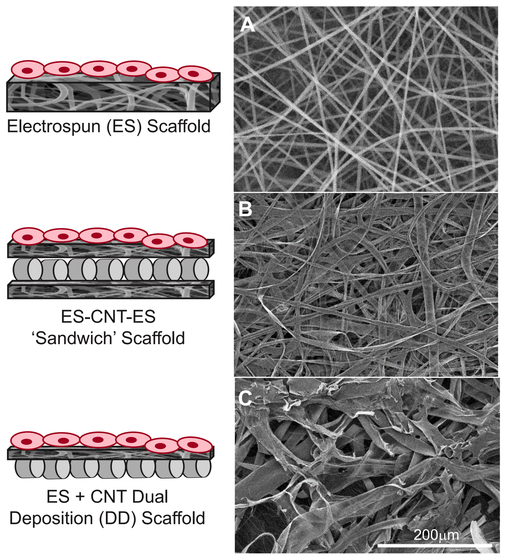

Figure 2: Conductive electrospun scaffolds *Scanning electron micrographs of PCL-gelatin electrospun scaffolds (A), "sandwich" scaffolds (B) and "dual deposition" scaffolds (C).*

Biodegradable electrospun scaffolds with Noggin coating

- Polycaprolactone (PCL) and gelatin were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol and extruded at a flow rate of 3mL/hr. 14kV was applied to electrospin.
- Noggin is a recombinant proteins that blocks the BMP pathway and promotes wound healing. We coated our electrospun scaffolds with Noggin to created a corneal bandage for the treatment of indolent corneal ulceration

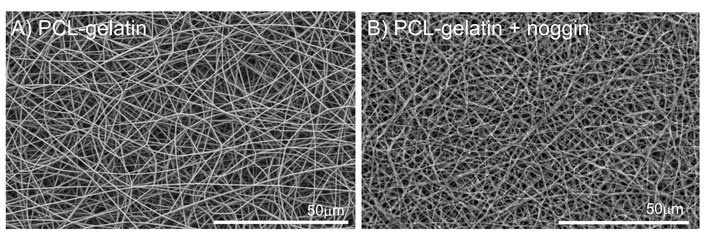

Figure 3: Biodegradable electrospun scaffolds with coating *Scanning electron micrographs of PCL-gelatin electrospun scaffolds (A) and PCL-gelatin scaffolds with noggin coating (B).*

Results: Collagen yarns

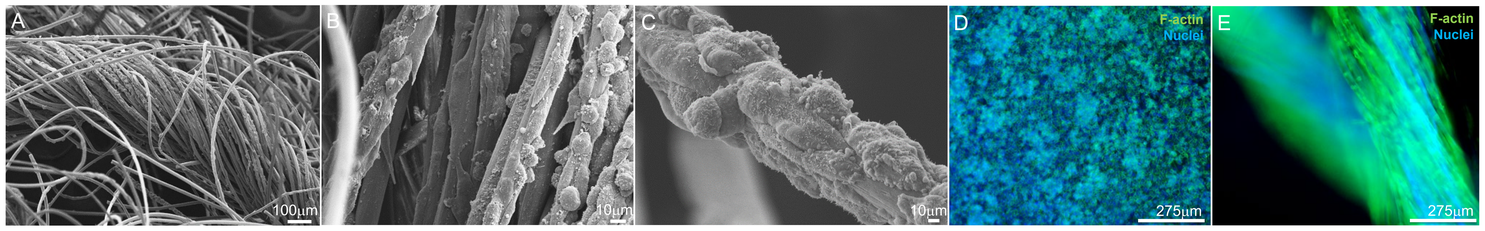

Figure 4: Collagen ringspun yarns *We evaluated the biocompatibility of natural collagen fibers ringspun into yarns. Scanning electron micrographs of NIH 3T3 cells seeded on collagen yarns after 7 days of culture (A-C). NIH 3T3 cells maintained on tissue culture plastic (D) and seeded on collagen yarns after 7 days (E) stained for cytoskeleton marker, F-actin (green) and nuclei (blue). Collagen yarns demonstrate excellent biocompatibility and sustain metabolic activity (data not shown). We plan to use collagen yarns in the future for several tissue engineering applications.*

Results: Electroconductive electrospun scaffolds

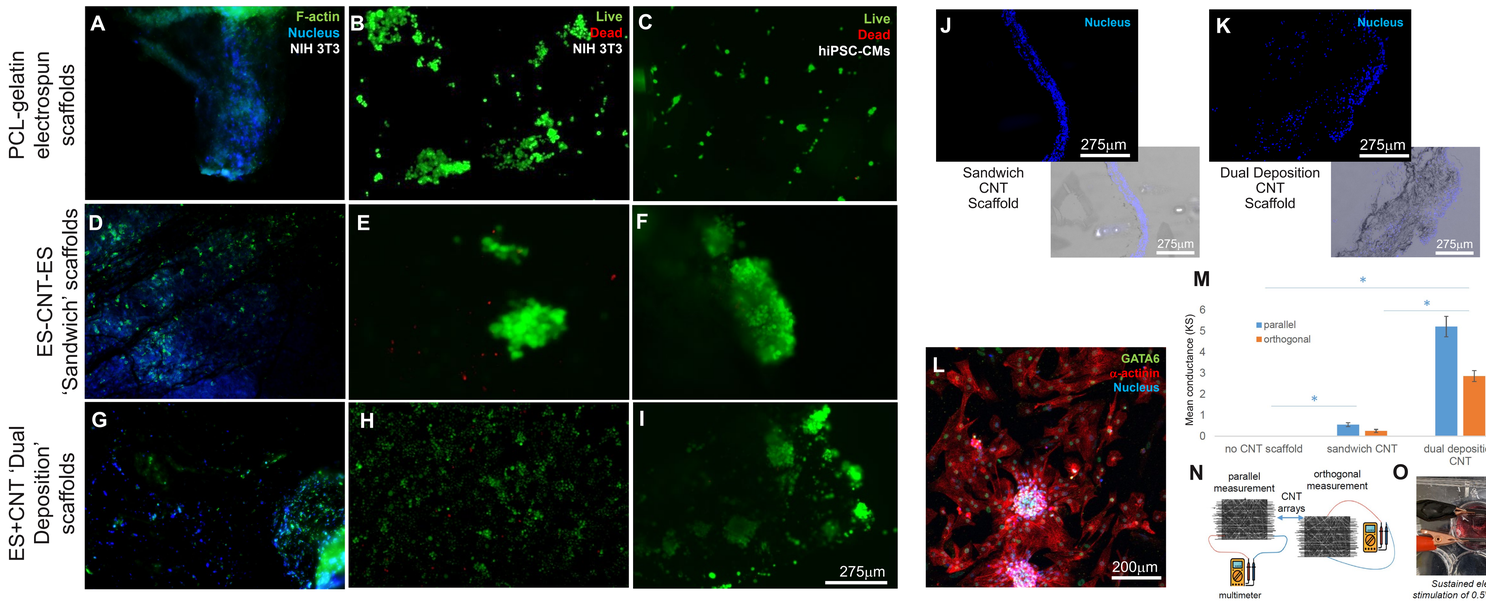

Figure 5: Electroconductive electrospun scaffolds *We sought to recapitulate the microenvironment of cardiac tissue by creating a conductive scaffold capable of propagating action potentials. Immunocytochemistry of cells seeded on electrospun scaffolds 7 days of culture (A-I) highlights the biocompatibility of our scaffolds. NIH 3T3 cells maintained on scaffolds (A-B, D-E, G-H) and human induced pluripotent stem cells (hiPSCs) maintained on scaffolds (C,F,I) for remain viable for 7 days. Biocompatibility was evaluated using cytoskeleton marker, F-actin, and Live/Dead assays of cells maintained for 7 days on electrospun scaffolds (A-C), Sandwich scaffolds (D-F) and Dual Deposition scaffolds (G-I). Paraffin sectioning (J,K) highlight NIH 3T3 cells migrating to the interior of the scaffold using nuclear stain and H&E imaging (insets J,K). hiPSC-derived cardiomyocytes (hiPSC-CMs) at day 14 post differentiation (L) were used for Live/Dead assays (C,F,I) and remain viable after 7 days. Scaffold conductivity as mean conductance (M) was determined using a multimeter to measure in the both the parallel and orthogonal directions (N). Electroconductive sandwich and dual deposition scaffolds were able to input an electrical stimulation to cells seeded on scaffolds sustained at 0.5V for at least 20min. We plan to use these scaffolds in the future to input an electrical stimulation to drive cardiac differentiation and induce a mature phenotype of hiPSC-derived cardiomyocytes.*

Results: Biodegradable electrospun scaffolds with Noggin coating

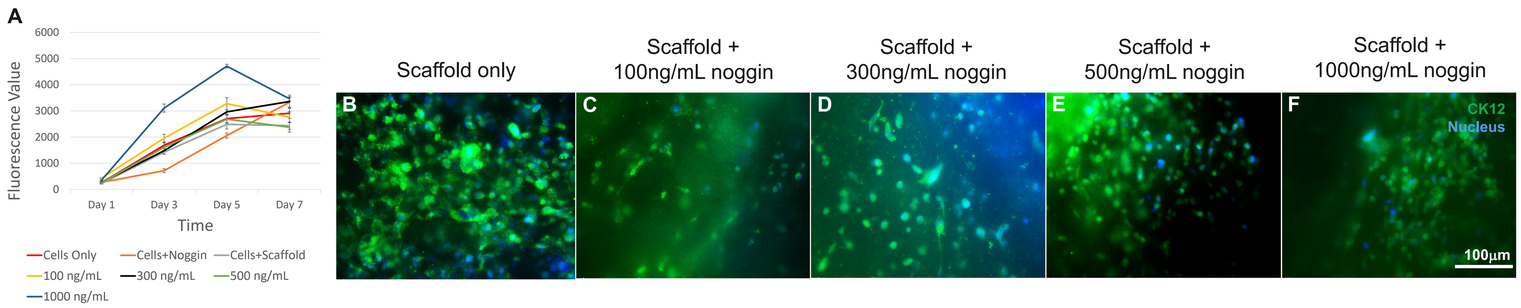

Figure 6: Biodegradable electrospun scaffolds with Noggin coating *We created PCL-gelatin electrospun scaffolds to use for corneal wound healing of indolent corneal ulcers. After coating scaffolds with Noggin to promote wound healing, we evaluated the biocompatibility using corneal epithelial cells (CECs). Metabolic activity was analyzed using AlamarBlue (A) which shows all scaffolds with varying concentration of Noggin promote CEC sustain CEC proliferation. Immunocytochemistry (B-F) shows that CECs maintain their functionality (CK12, green) when maintained on scaffolds coated with varying concentrations of Noggin for at least 7 days. We will continue to optimize our scaffolds to determine the best concentration of Noggin to apply to support wound healing.*

Conclusions

- We are able to successfully recapitulate the 3D microenvironment of native tissues using synthetic biomaterials.
- Our scaffolds are demonstrated to be highly biocompatible.
- We plan to continue our work in wound healing, cardiac tissue engineering and corneal tissue engineering.

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