Pneumatic-drive Fiber-Robot Scaffold for Three-Dimensional Dynamic Tissue Culture

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Statement of Purpose: With the recent emergence of stem-cell-based therapies for the treatment and regeneration of damaged tissues. The need for dynamically active scaffolds for mimicking in-vivo conditions of the human body is profound, as mechanical stimulations throughout cell cultures have been shown to elicit important changes in cellular differentiation, tissue growth, and gene expression[1]. In this study the developed fiber-based scaffold consisted of either, ultra-high molecular weight polyethylene (UHMWPE) or Nylon-6 braided fabric, which covered the elastomeric bladder of the device. Therefore, this study investigated the ability of the dynamic fiber robot scaffold to efficiently act as a dynamic tissue scaffold to promote the cell adhesion and proliferation of mouse fibroblasts under conditions of mechanical stress.

Methods: Two 0.9mm diameter fiber robot scaffolds made up of either UHMWPE(Dyneema) (Koninklijke DSM N.V. DSM Protective Materials, Greenville North Carolina) or Nylon-6 braided sheath were seeded with NIH 3T cells at a density of 10^6 per cm^2 and analyzed for cell viability under controlled and static conditions three times over a 12-day period using a Live/Dead cell viability assay. Media was changed every other day during this 12-day period. On the twelfth day, immunocytochemistry was performed on the Dyneema samples in order to confirm the presence of mouse fibroblasts on the seeded scaffold, specifically analyzing the presence of F-actin filaments (Phalloidin staining). Additional fiber robot scaffolds, made from the same materials, were then seeded with NIH 3T3 cells at the same cell seeding density and incubated for a period of eight days, and media was changed every other. A Live/Dead Cell Viability Assay was then performed before the two samples began an 18.5-hour dynamic study. The samples were then placed in custom T75 flasks and mechanically stimulated using a computerized pneumatic system that inflates and deflates the fiber robot at a frequency of 0.5 Hz.

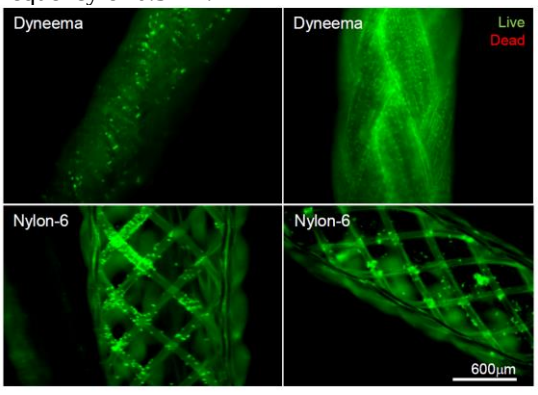

Figure 1: NIH 3T3 cells remain viable after 12 days when maintained under static conditions on Dyneema and Nylon-6 scaffolds.

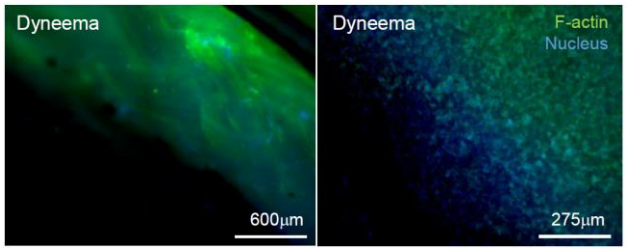

Figure 2: F-actin (green) staining of NIH 3T3 cells after 12 days of culture when maintained under static conditions on Dyneema.

Results: Initial cell viability for the fiber robot scaffolds under static conditions showed cell biocompatibility, as the cellular proliferation, and adhesion of the NIH 3T3 cells was observed from a Live/Dead assay performed after cells were maintained on the Dyneema and Nylon-6 construct for 12 days (Figure 1). Cells adhered and proliferated along the lengths of the fibers within the scaffold, as seen from the cytoskeletal marker, F-actin after Phalloidin staining

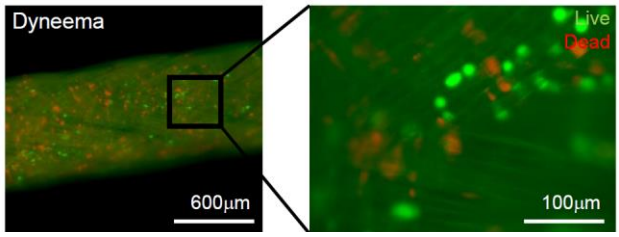

Figure 3: Viability of NIH 3T3 after 18.5hrs of dynamic culture at room temperature on Dyneema structures.

(Figure 2) after 12 days of culture. Additionally, the fiber robot-maintained biocompatibility after an 18.5-hour period of mechanical stimulation at 0.5 Hz. Approximately 60% of NIH 3T3 cells-maintained viability as observed from a simple Live/Dead assay (Figure 3). It should be noted that the dynamic stimulation was conducted at room temperature. Cells maintained on Nylon-6 structures showed significantly better cell viability and impressive cellular proliferation even when under the uncontrolled environmental conditions of a biosafety-hood.

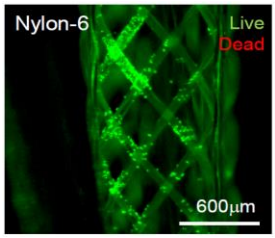

Figure-4: Viability of NIH 3T3 after 18.5hrs

Conclusion: Our findings indicate that the Fiber-Robot scaffold is biocompatible and supports dynamic culture for sustained time periods. We plan to use the Nylon-6 fiber-robot scaffold to study mechanotransduction during development with a stem cell model in the future.

References:

1) PC Dartsch. Basic Res Cardiol, 1989; 84: 268-281.