Hydrogel Thickness and Nanofiber Connectivity Influence Cell Alignment and Morphology in Hydrogel-Nanofiber Composites

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Statement of Purpose:

Biophysical cues play an important role in influencing cell behavior. For example, contact guidance between cells and aligned nanofibers triggers guided cellular elongation, migration, differentiation, and extracellular matrix formation.[1,2] Although cell-nanofiber interactions have been extensively studied, the attenuation (or enhancement) of contact guidance between cells and nanofibers embedded in an elastic medium is not well understood. The goal of this study is to investigate the effects of hydrogel spacing between cells and nanofibers and nanofiber-hydrogel connectivity on the ability of cells to sense nanofiber biophysical signals. Single layer arrays of aligned polycaprolactone (PCL) nanofibers were embedded in methacrylated (MA) gelatin (GelMA) hydrogels to create Thin (~40-60 µm) or Thick (~80-100 µm) hydrogel-nanofiber composites. PCL nanofibers were also formed with MA(+) or without MA(-) methacrylated poly(ethylene glycol) (PEG) to investigate the effects of connectivity (via MA kinetic chain growth entanglement) post hydrogel/nanofiber photopolymerization. To evaluate contact guidance of embedded nanofibers, mesenchymal stem cells (MSCs) were cultured atop Thin and Thick nanofiber-hydrogel composites with and without nanofiber MA modification. It is hypothesized that hydrogel thickness attenuates and nanofiber-hydrogel connectivity enhances nanofiber-mediated contact guidance.

Methods:

An *in vitro* model was created to assess the effects of hydrogel thickness and nanofiber connectivity of MSCs cultured atop hydrogel-nanofiber composites. Plastic collection frames were used to gather aligned, electrospun PCL fibers on a single 2D plane. Using the thermoreversible properties of gelatin, the frames were dip-coated in gel (liquid) at 40°C (above GelMA melting temperature) and subsequently polymerized with UV light (10 mW/cm^2, 90 s per side). MSCs (3,000 cells/cm^2) were seeded on top of hydrogel-nanofiber composites for 1 day. Samples were fixed with formalin and stained with Alexa Fluor 568 Phalloidin (actin, cell body) and Hoechst (nuclei). Samples were imaged with a confocal microscope to image cell bodies (red), nuclei, (blue), and encapsulated PCL nanofibers (blue). Single-cell image analysis (ImageJ) was then performed to quantify Area, Aspect Ratio, Circularity, and directed alignment of MSCs cultured atop nanofiber-hydrogel films consisting of Thin and Thick hydrogel spacing with (MA(+)) and without (MA(-)) connectivity.

Results:

Representative confocal images of single MSCs atop Thick (Fig. 1A) and Thin (Fig. 1B) composites qualitatively demonstrate that hydrogel thickness reduces nanofiber-mediated contact guidance. This was supported by observing decreased alignment (angle between the line bisecting the longest length of the cell and the nearest underlying nanofiber) of MSCs on Thick relative to Thin hydrogel groups (Fig. 1C). Within the MA(-) groups, MSCs on the Thin hydrogel group had higher Area (Fig. 1D), higher Aspect Ratio (Fig. 1E), and lower Circularity (Fig. 1F) than the Thick gel group. This data shows that a thinner gel layer will favor conditions that lead to a greater degree of cell alignment and spreading along the encased fibers. Within the MA(+) groups, the Thin gel group had higher Aspect Ratio and lower Circularity than the Thick gel group. Within the Thick gel groups, the MA(+) group had a significantly higher Area, higher Aspect Ratio, and lower Circularity than the MA(-) group. Within the Thin gel groups, the MA(+) group had a significantly lower Circularity than the MA(-) group. This data indicates that nanofiber connectivity favors conditions that lead to a greater degree of cell alignment and spreading along the encased fibers. By further evaluating the mechanosensing ability of cells seeded on these 2-Dimensional constructs as well as expanding experimental conditions to 3-Dimensions, these GelMA and PCL nanofiber constructs can be utilized for applications of Tissue Engineering to regenerate aligned tissue types such as peripheral nerve, skeletal muscle, and aligned connective tissue.

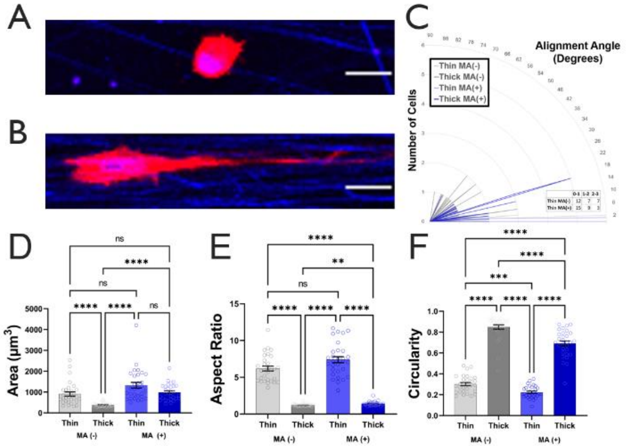

Figure 1: Representative confocal images of single MSCs seeded on (A) Thick and (B) Thin hydrogel-nanofiber composites. (C) Rose plot of Angle of Alignment between MSCs and aligned nanofibers on 4 test groups (Thin, Thick, MA(+), MA(-)). Bar graphs show effects of hydrogel thickness and nanofiber connectivity on MSC (D) Area, (E) Aspect Ratio, and (F) Circularity. At least 30 cells were analyzed per group. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$

References:

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