Supplemental Information for

Understanding the Role of ECM Protein Composition and Geometric Micropatterning for Engineering Human Skeletal Muscle

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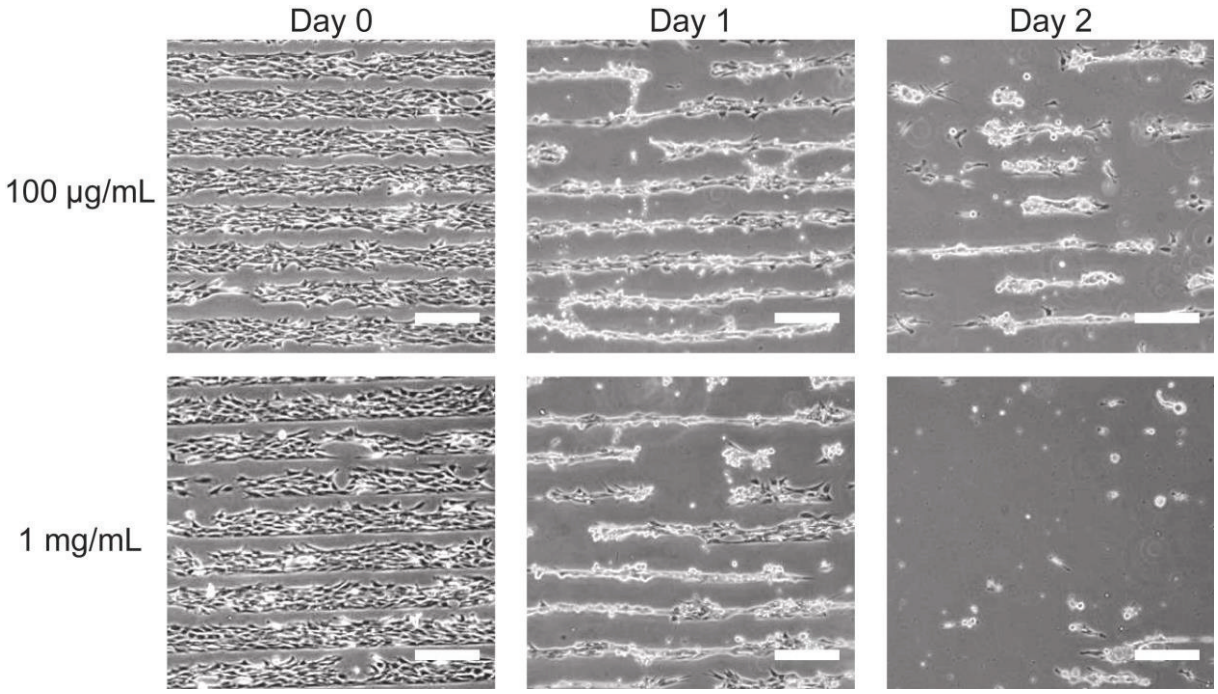
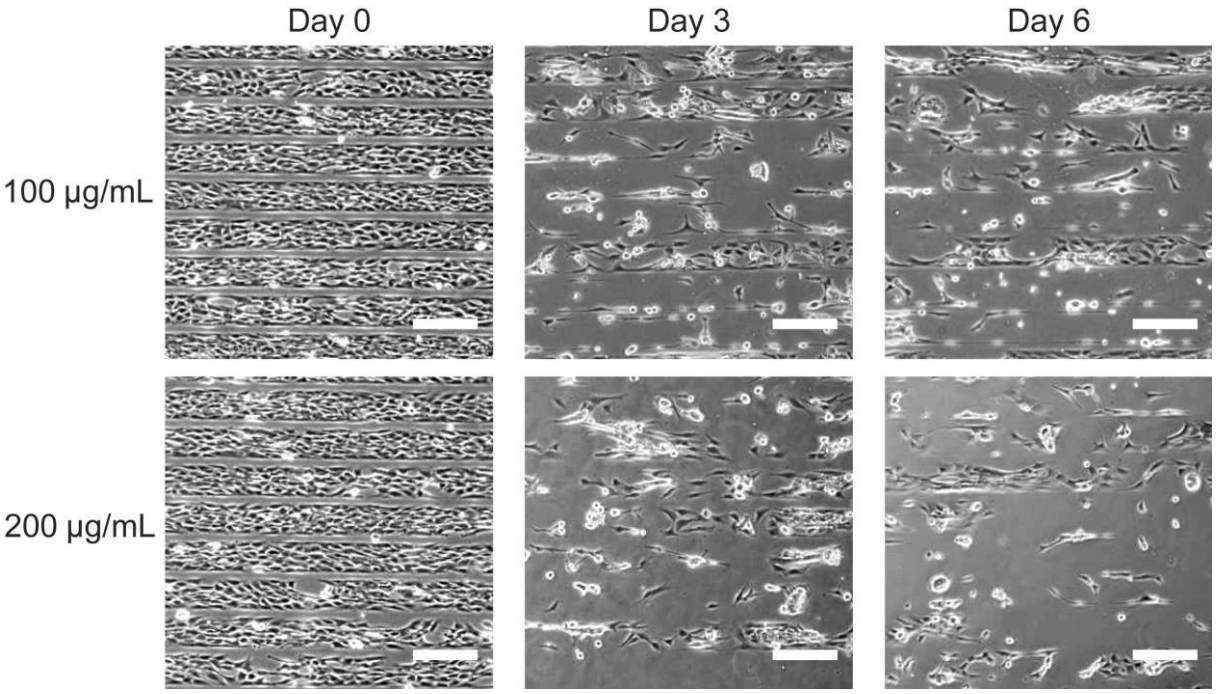
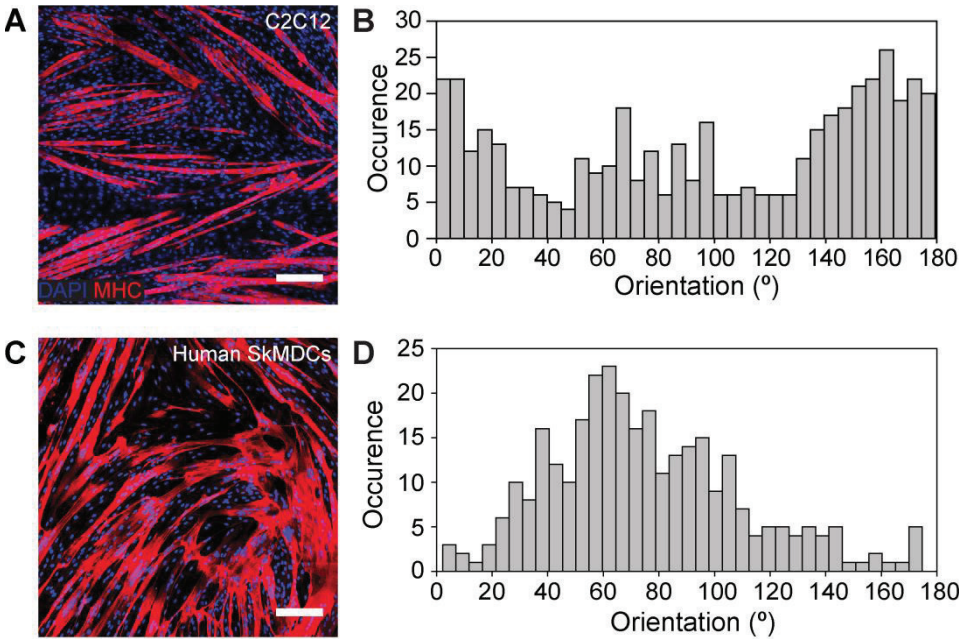
This PDF file includes:



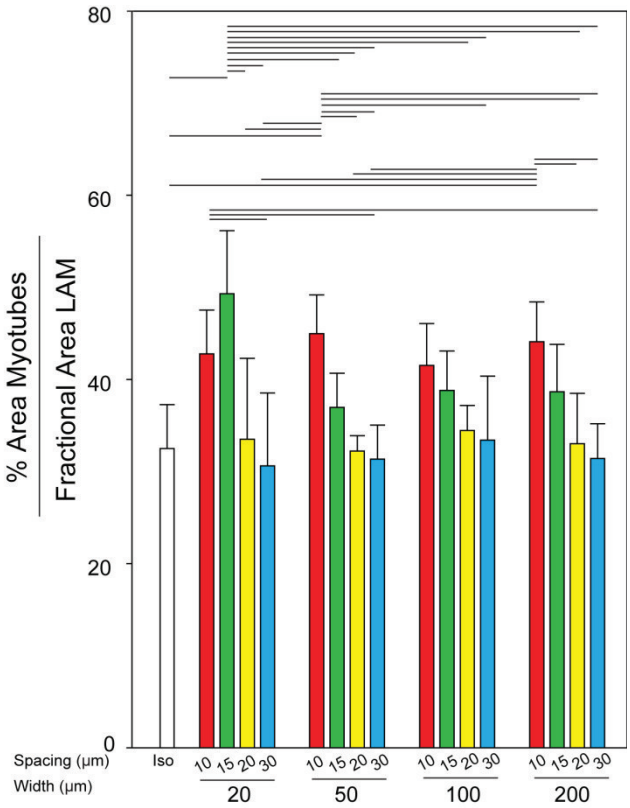
Supplemental Figure 1: C2C12 myoblasts were switched to differentiation media after reaching confluence on 100x20 micropatterned lines of Col I (Day 0). At both low (100 µg/mL) and high (1 mg/mL) Col I concentrations, myoblasts began peeling off of patterned Col I before fusing to form myotubes (Day 1, Day 2). Scale bars 200 µm.



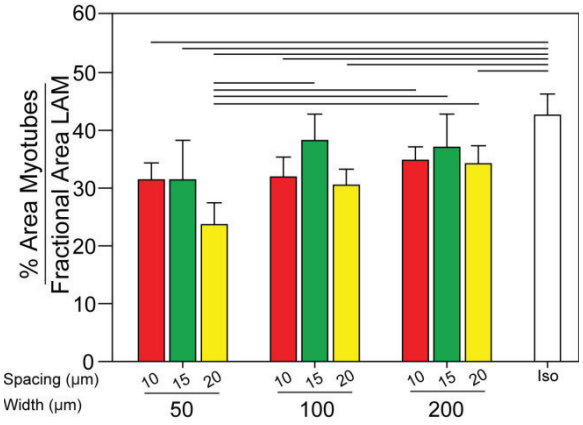
Supplemental Figure 2: C2C12 myoblasts were switched to differentiation media after reaching confluence on 100x20 micropatterned lines of Col IV (Day 0). At both low (100 µg/mL) and high (200 µg/mL) Col IV concentrations, myoblasts began peeling off of patterned Col IV before fusing to form myotubes (Day 3, Day 6). Scale bars 200 µm.



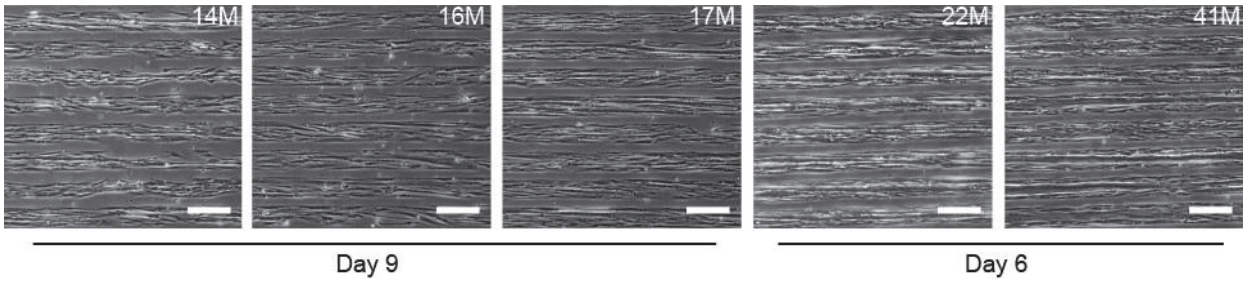
Supplemental Figure 3: Myotubes formed on isotropic coatings of LAM. **(A)** C2C12 and **(B)** Cook's human SkMDC myotube formation on isotropic LAM coated coverslips. Histograms of myotubes on isotropic LAM for **(C)** C2C12 cells and **(D)** human SkMDCs shows little to no preferential orientation or alignment of myotubes. Scale Bars 200μ m.



Supplemental Figure 4: C2C12 myotube formation measured by percent area myotubes and normalized for percent area of patterned LAM. Significantly more myotubes formed on patterns that allowed C2C12 myoblasts to fuse across line spacings rather than restricting myotube fusion to occur only on μCP lines. Additionally, guidance cues from μCP lines appear to increase myotube area/LAM area as several of the conditions have significantly more myotube formation than the isotropic control. n = 6. Lines represent $p<0.05$.



Supplemental Figure 5: Human SkMDC myotube formation normalized for percent area μCP LAM for each pattern. Unlike C2C12s, human SkMDCs form higher percent area myotubes/area LAM on isotropically coated LAM. Additionally, myotubes formed on 50x20 patterns had significantly less differentiated myotubes even after normalization for patterned area. Myotubes did not fuse across 10 and 15 μm line spacings, so there significantly higher myotube formation was not observed on these patterns when normalized for area patterned LAM as observed with C2C12 myotubes. N = 5 for 50x10 and 100x10 patterns. N = 6 for all other conditions. Lines represent $p<0.05$.



Supplemental Figure 6: Phase images of human SkMDCs on μCP 100x20 lines of LAM. Different cell isolations are represented in each image, with donor age and gender represented in the upper right. Degree of myotube formation varies from sample to sample, but even it phase it is apparent that samples with myotube formation generally have wide, long myotubes that take up most of the patterned line area, just as the samples from the 17F donor that were quantified in the other sections of this paper. It appears that this increased alignment and restriction to patterned lines is a qualitative trend for human SkMDCs isolated by Cook Myosite. Scale bars 200 μm.