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Supporting Information

Direct cellular organization with ring-shaped composite polymer and glass substrates for urethral sphincter tissue engineering

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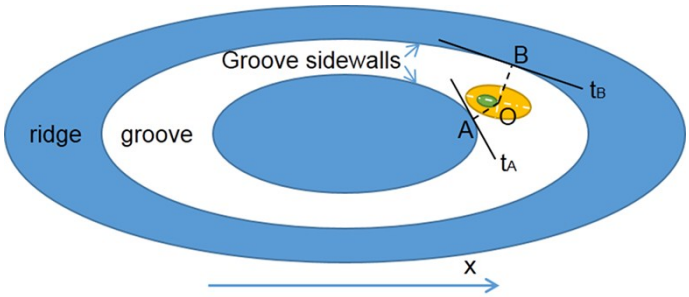

Fig. S1. Diagram illustrating the definition of groove direction and groove curvature. Groove direction was defined as the tangential direction of the point on groove sidewalls that was nearest to cell center. Groove direction is the direction of line t_A in this case (|OA|<|OB|). Groove curvature is the curvature of groove sidewall on point A.

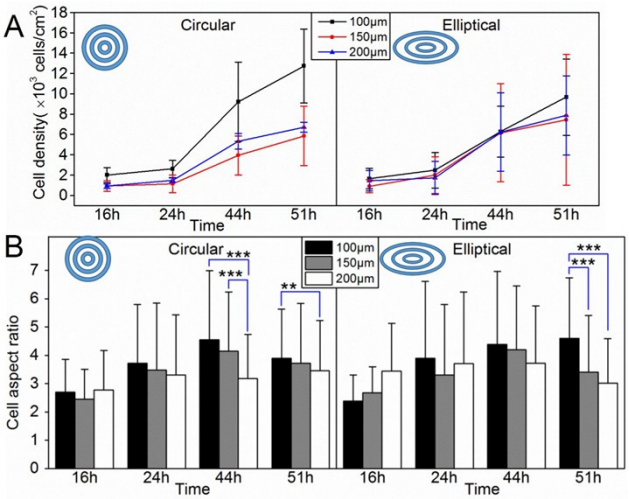

Fig. S2 Cell density and aspect ratio calculated and presented as mean ± standard deviation (SD). (A) Cell density with respect to culture time on circular and elliptical substrates. No statistically significant difference of cell density was found among all dimensions of grooves of both circular and elliptical substrates at any given examine time point ($p > 0.06$ for all cases), which means neither dimension nor pattern of ring-shaped substrate affect myoblast proliferation. (B) Histograms showed statistical comparison of cell aspect ratio with constraints dimensions for cells on circular and elliptical substrates with 100, 150 and 200 μm wide grooves at 16, 24, 44 and 51 h time point.

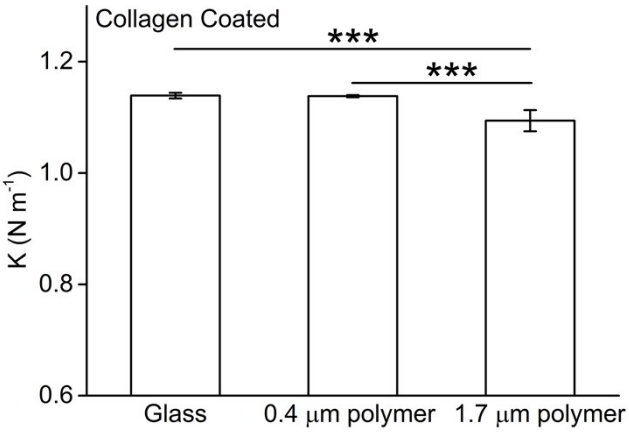

Fig. S3. AFM indentation of collagen coated glass, 0.4 μm and 1.7 μm thick polymer. Statistical analysis of the slope (K) of force versus indentation depth inferred that stiffness of 1.7 μm polymer is significantly lower than that of 0.4 μm polymer and glass. But stiffness of 0.4 μm polymer showed no difference compared to that of glass.

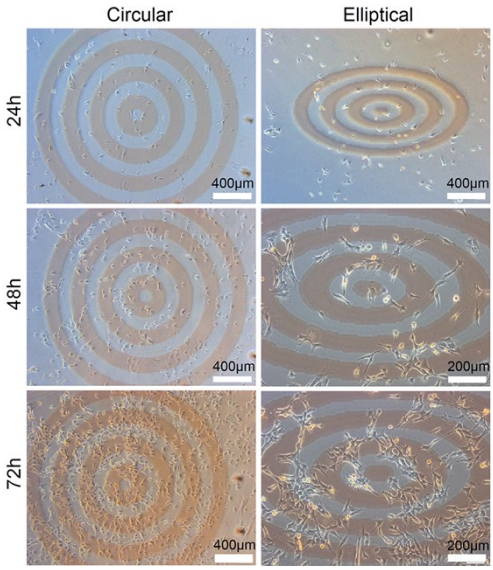

Fig. S4. Phase contrast images of cells cultured on circular and elliptical patterned substrates with 0.4 μm deep and 150 μm wide grooves at 24, 48 and 72 h after culture.

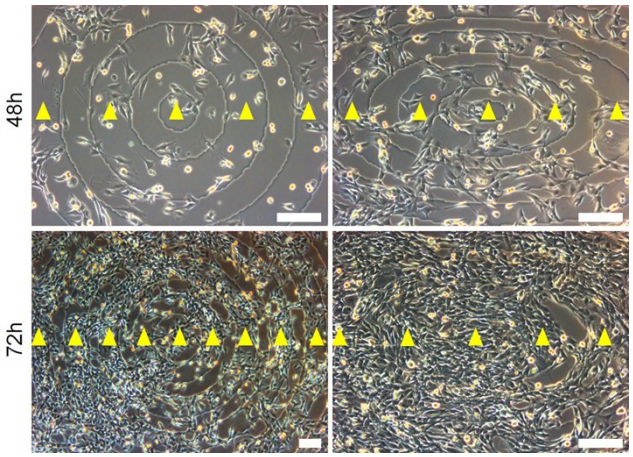

Fig. S5. Phase contrast images of C2C12 proliferating on 1.7 µm grooved glass substrates. Cells showed no preference for migrating inside and proliferating in grooves for both circular (left panel) and elliptical (right panel) substrates. Ridges were marked with yellow triangle. Scale bar is 200 µm.

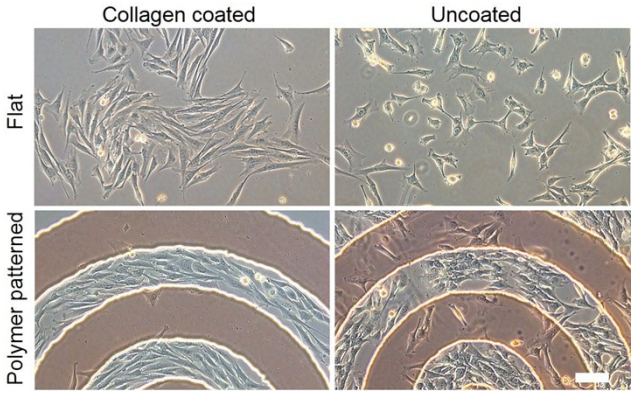

Fig. S6. Phase contrast images of C2C12 cultured on Flat and polymer patterned glass substrate with (left) and without (right) collagen coating. Cells were more elongated on collagen coated than uncoated substrates.

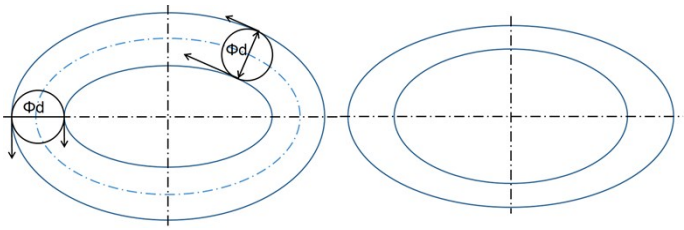

Fig. S7. Diagram illustrating elliptical grooves with parallel groove sidewalls with fixed width (left) and unparallel groove sidewalls with varied width (right).

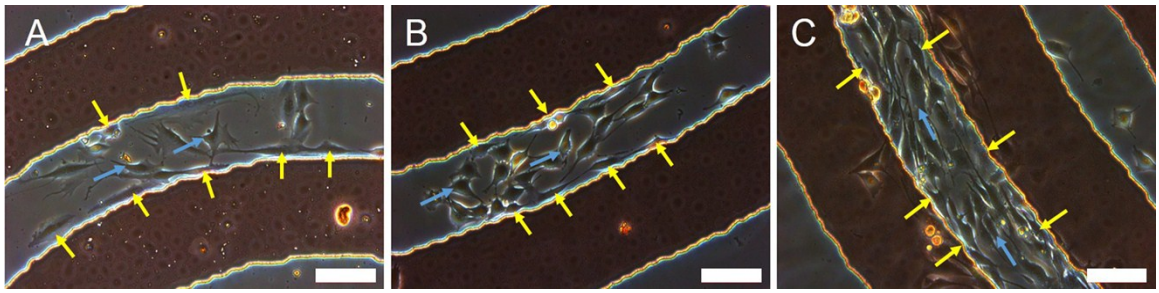

Fig. S8. Phase contrast images of C2C12 orientation with respect to groove direction at low (A), middle (B) and high (C) level cell-cell contacts. Degree of cell alignment increased as degree of cell-cell contacts increased. Cells also showed a position-dependent alignment with cells near the boundaries of the groove align parallel to groove directions at all level cell-cell contacts (yellow arrows) and cells in the middle region of the groove randomly oriented at low level cell-cell contacts while reoriented along groove directions at high level cell-cell contacts (blue arrows). Scale bar is 100 μm.