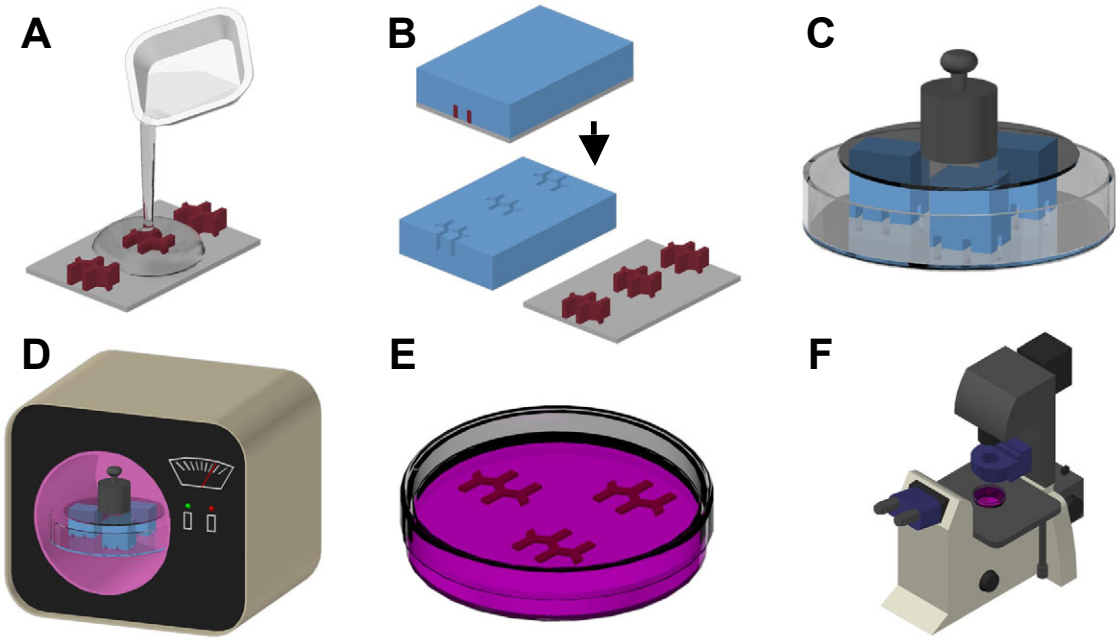

Figure S1. Plasma lithography for surface patterning. (A) Cast liquid PDMS polymer onto 3D microstructure to create shielding mold. (B) Remove cured PDMS from master surface. (C) Shielding molds placed onto surface with weight to ensure conformal contact. (D) Chemical patterning by plasma treatment. (E) Seed with cells. (F) Observation.

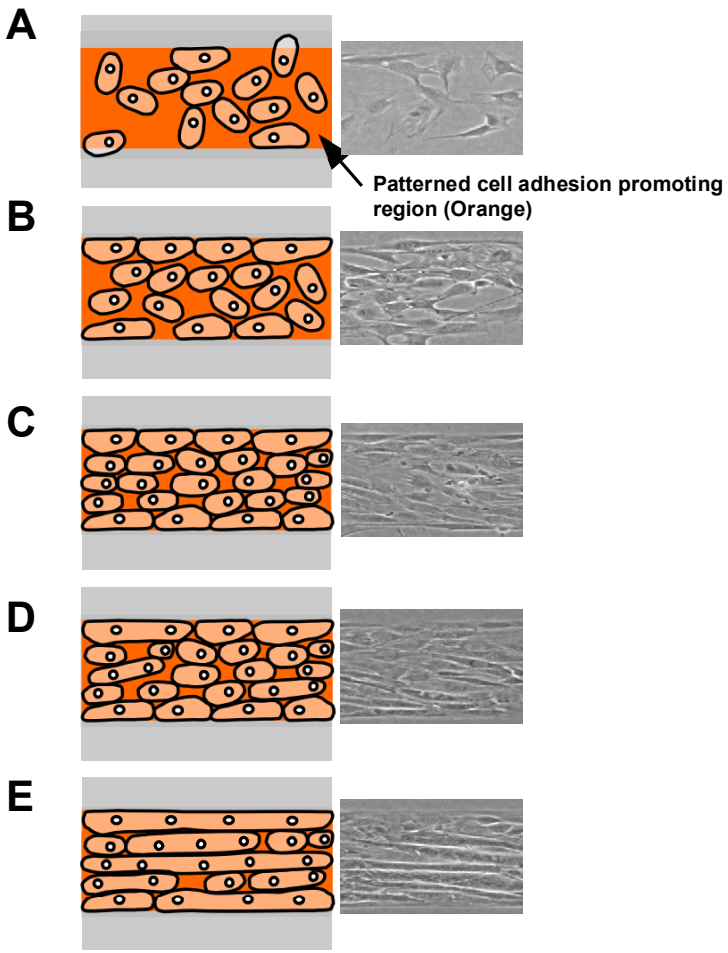

Figure S2. Process of alignment on a line pattern. (A) Initially cells adhere preferentially to patterned areas but are not highly aligned, except at pattern edges. (B) Cell density and alignment increases. Cells also preferentially migrate onto cell friendly areas and remain there. (C) When cells are 80-90% confluent, media is changed to initiate differentiation. (D) Cells begin to fuse and alignment of myotubes increases from the edge areas. (E) Cells continue to fuse and alignment increases further.

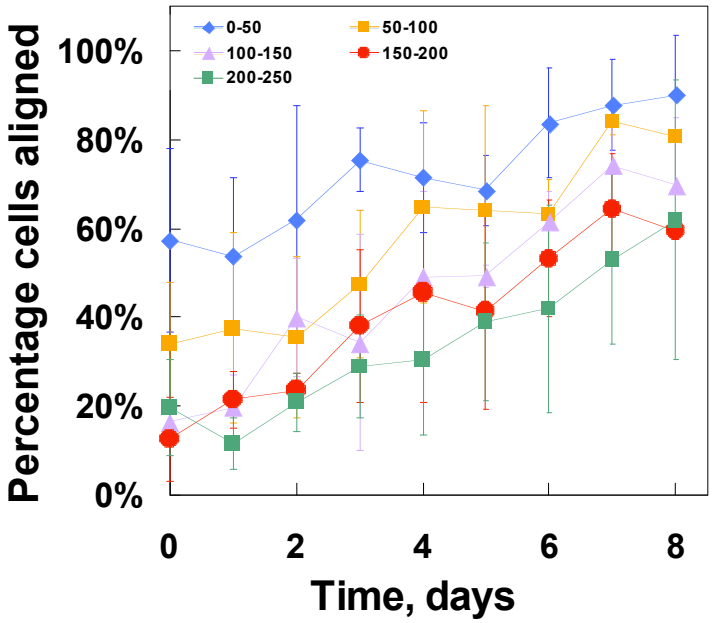

Figure S3. Spatiotemporal distribution of myoblasts aligned on a line pattern that is 500 μm in width. The cells are grouped by the distance (in μm) from the boundary of the line. Cells near the boundary are indicated as 0-50 while 200-250 represents cells at the center of the line. Data represent mean ± standard deviation.

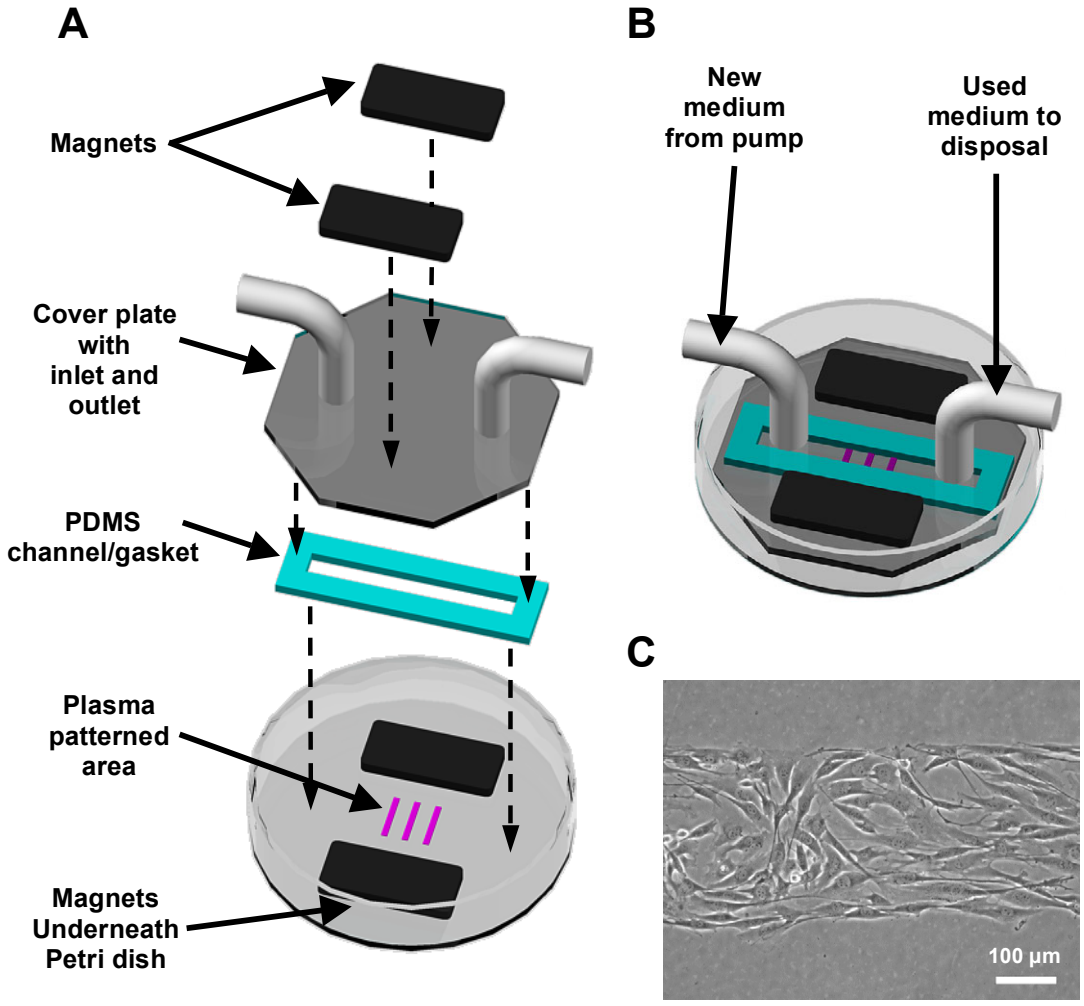

Figure S4. Cell growth inside microchannels. (A) Formation of a microchannel for controlling media supplied to C2C12 myoblasts. The substrate is first patterned by plasma lithography as described in the text, and is then encapsulated inside a channel connected to a source of media. (B) Completed channel. Media is supplied at 0.2 - 0.6 ml/hr in one of two ways. The first being continual supply of fresh medium and the second being recirculation of medium. In both instances a peristaltic pump (Fisher Scientific Ultralow Flow Variable-Flow Peristaltic pump) was used for pumping media and the flow rates were selected to ensure that shear forces would not affect the cellular behavior. (C) C2C12 myoblasts aligning but not fusing inside a microchannel when fresh differentiation media is continuously perfused through the system.

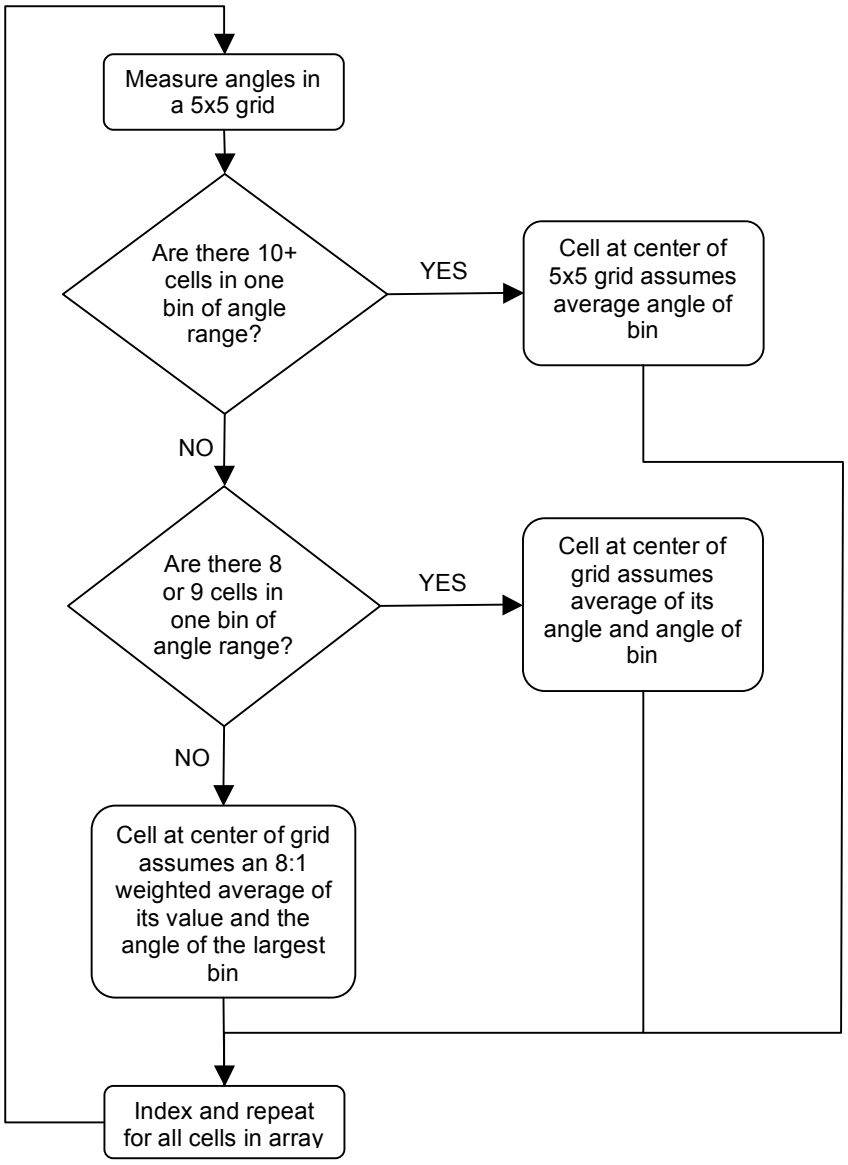

Figure S5. Cellular automata modeling. Cell alignments were simulated in MATLAB using a program designed to model cell behavior based upon relationships between a small neighborhood of cells. The program first examines the angles of cells in a 5 x 5 grid and places the cells into groups of 10^{o} bins. The program then counts how many cells are in each bin. If ten or more cells fall into the same angle bin then the cell at the center of the 5 x 5 grid assumes the average angle of those ten (or greater) aligned cells +/- a small random increment of angular motion. Otherwise, if between eight and nine cells fall into the same angle bin then the cell at the center of the 5 x 5 grid assumes the average of the angle of the aligned block of cells and its current cellular angle +/- a small random increment of angular motion. Otherwise if a bin of cells has less than eight cells in it, then the cell at the center of the 5 x 5 grid assumes an 8:1 weighted average of its own alignment (8) and the average alignment of the cells with the greatest number in their alignment bin (1) +/- a small random increment of angular motion. This is then repeated for every cell in the array once per time step and cellular angle is mapped to a color and displayed. The algorithm for non-fusing cells is for general alignment to neighboring cells without a decision based upon fusion or a high degree of alignment. During each time step, the central cell assumes a 2:1 weighted average of the largest aligned bin of cells (2), and the value of the central cell (1).