Supplementary Data

Experimental Methodology for Data Presented in Figure 4

Collagen I was obtained from rat tail tendon by solubilization in 0.01 M HCl at 4°C overnight. Subsequently, the solution was centrifuged for 45 min at 30,000 *g* and the supernatant was removed, frozen, and lyophilized. Stock collagen solutions of 0–20 mg/mL were obtained by reconstitution of lyophilized collagen in 0.01 M HCl. Multiple stock solutions were prepared so that hydrogels of varying concentration could be fabricated while maintaining a 2:1 dilution factor for each.

Collagen hydrogels were fabricated by combining acidic collagen with a concentrated buffer (10× DMEM, no supplements; Sigma), neutralization agent (1 N NaOH; Fisher), and physiological buffer (1× DMEM supplemented with glucose, sodium bicarbonate, and L-glutamine; Sigma). The volume fraction of each component was as follows: acidic collagen = 0.5, 10× DMEM = 0.1, NaOH = kNaOH = 0.0107–0.0267 (control variable), and 1× DMEM = 0.373–0.389 (remaining volume).

Three milliliters of hydrogels was prepared by combining all components over ice and stirring with a spatula. The data in Figure 4 were obtained at room temperature (23°C) using a glass pH electrode (Thermo) submerged in each hydrogel.