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**Fabrication of hydrogel substrates with stiffness step variations using controlled surface wettability** MD. MAHMUDUR RAHMAN, DONGHEE LEE, SANGJIN RYU, University of Nebraska-Lincoln — Living cells can respond to changes in the stiffness of the surrounding matrix. Well-known examples include the durotaxis of motile cells and the stiffness-dependent differentiation of stem cells. Such mechanobiological behaviors of living cells have been investigated on hydrogel substrates of which the compliance is either static or varying in one direction. Although various techniques have been developed to fabricate hydrogel substrates of controllable stiffness distributions, however, the fabricated substrates have only hydrogel regions of varying stiffness, lacking regions of static stiffness. Therefore, it has been difficult to compare cells' responses to static stiffness and varying stiffness under the same culture condition. Thus, we aim to fabricate polyacrylamide gel substrates consisting of alternating regions of static stiffness and stiffness gradient. For controlled positioning of gel solutions with different relative concentrations of acrylamide and the crosslinker, we generated superhydrophilic regions surrounded by hydrophobic barriers on glass and then filled the regions with the gel solutions. When sandwiched by another glass surface, the gel solutions experienced limited mixing only at interfaces, which created stiffness gradients between static stiffness regions.

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