

Developing three-dimensional whole cell based biosensor format with SU-8 microstructures

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Creating three-dimensional (3-D) whole cell based biosensor (CBB) format is of both theoretical and practical importance as three-dimensionality provides cells with characteristic topographical cues. In such cellular microenvironments, cells differentiate into specific phenotype and maintain specific functions that are usually impossible under two-dimensional (2-D) culture conditions. We have previously evaluated collagen hydrogel and packed Cytodex microbead array as 3-D CBB format and found significant differences between SH-SY5Y cells in 2-D layer and those in 3-D configuration in terms of resting membrane potential establishment and voltage-gated calcium channel function development. We are now extending these efforts to create 3-D CBB format with SU-8 microstructures. SU-8 microwell patterns were fabricated with well diameters of 15 μm , 50 μm and 100 μm by UV photolithography. We found that 100- μm microwell patterns were the most suitable structures for SH-SY5Y cell integration among the dimensions tested. We then fabricated channel-connected well network patterns with a well diameter of 100 μm , channel length of 90 μm and channel width of 10 μm . With a combination of polyethylene glycol stamp inking of the top pattern surface and laminin coating of the well network structure, we have achieved a topographically patterned neuronal network of SH-SY5Y cells. We next analyzed resting membrane potential establishment of SH-SY5Y cells on flat SU-8 substrates and well network patterns using confocal microscopy and a potentiometric fluorescent dye TMRM. With dcAMP and BrdU as differentiation inducing agents, cells on flat SU-8 substrates developed a resting membrane potential of -15.0 ± 21.8 mV ($n = 112$), which tended to decrease to -9.8 ± 20.1 mV ($n = 104$) on day 13 into differentiation ($P > 0.05$). Cells on well-network patterns developed a resting membrane potential of 27.2 ± 26.3 mV ($n = 51$) on day 5 into differentiation, which remained at the same level of -26.2 ± 21.8 mV ($n = 62$) on day 13 into differentiation ($P > 0.5$). Cells on well-network patterns had more negative resting membrane potential values than on flat SU-8 substrates, on either day 5 or day 13 into differentiation ($P < 0.01$). These results clearly suggest that well network patterns, or topographically patterned 3-D SU-8 substrates, were more favorable formats for promoting SH-SY5Y cell resting membrane potential establishment than flat SU-8 substrates, demonstrating the potential significance of using 3-D structure as CBB format.