P0366 / #4709

Topic: AS04 Neurons and Glia: Physiology and Inter-Cell Communication

BACTERIAL CONTACT INDUCES MORPHOLOGICAL AND FUNCTIONAL CHANGES IN PRIMARY NEURAL CORTICAL CELL CULTURES

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The interaction of bacteria with various somatic cell types is an exciting emerging field. Despite the known effects of microbiota on the gut-brain axis, very little is known about the direct interactions that bacteria could have with neurons, both in terms of molecular mechanisms and information transfer. In order to study these communication mechanisms, this study designs an in vitro model to co-cultivate microbiota-bacteria *Lactiplantibacillus plantarum* with neural cortical cells and analyzes the effects of this process in both populations. Here, we show how bacteria and neurons can be co-cultured, and demonstrate a novel integrated platform that facilitates the analysis of neuronal-bacteria communication. The results we obtained showed that *L.plantarum* is capable of adhering to the surface of the neural culture and the amount of attached bacteria increases with co-culture time. In addition, neural co-cultured cells show morphological and functional changes in the expression of key proteins in neuroplasticity such as Synapsin I and pCREB. Finally, using real-time optical (calcium signaling) readouts, we show that neural cells react to the co-culture with bacteria by increasing cytoplasmatic Ca^{2+} signaling. Our proof-of-principle data reveal crosstalk between bacterial and neural co-cultured cells and illustrate a novel example of cross-kingdom communication between highly diverse cell types. The ability to eavesdrop on information passing between these two very different levels of biological organization will facilitate insight into evolutionary cell biology and could impact the understanding of brain-bacteria communication for diagnosis of neuronal states in health and disease.

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EFFECT OF 3D SYNTHETIC MICROSCAFFOLD "NICHOID" ON THE MORPHOLOGY OF CULTURED HIPPOCAMPAL NEURONS AND ASTROCYTES

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The human brain is the most complex organ in biology, being composed of an extraordinary number of synapses. Considering that the brain pathologies are bound to rise, there is an essential need to establish an effective in-vitro system of the Central Nervous System that could be applied to test new therapeutic avenues. To this aim, we set up a new model of hippocampal neurons and astrocytes co-culture taking advantage of the Nichoid technology, a 3D scaffold microfabricated by two photon laser polymerization, to generate brain micro-tissues of 30 μm thickness. After 21 days in-vitro, by confocal microscopy, we morphologically characterized the co-cultures comparing 2D and 3D conditions. We observed that astrocytes as well as neurons had become well-differentiated and colonized the entire volume of the Nichoid. This was further elaborated with the use of Drebrin, PSD-95, and Synaptophysin antibodies that labelled the majority of neurons, both in the 2D as well as in the 3D co-cultures. Interestingly, in the Nichoid, astrocytes displayed a more physiological morphology, closer to the in-vivo condition, appearing more starry compared to 2D cultures. Lastly, using Scanning Electron Microscopy, we found that neurons co-cultured with astrocytes in the 3D environment showed more dendritic spine protrusions compared to the 2D culture, suggesting they could be more prone to form connections. Our results show that the Nichoid can be used as a 3D device to investigate the structure and morphology of neurons and astrocytes in-vitro as well as the complex cell-cell interactions within the brain. In addition, it may serve as a tool to study mechanisms governing synaptic plasticity/dysfunction and to drug discovery.

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