Aragonite Crystalline Biomaterials as Bioactive and Instructive Microenvironments for Neural Development

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INTRODUCTION: Identifying scaffolds supporting in vitro reconstruction of active neuronal tissues in their three-dimensional (3D) conformation is a major challenge in tissue engineering. We have previously shown that aragonite coral exoskeletons support the development of neuronal tissue from hippocampal neurons and astrocytes[1-4]. Here we investigated the cues that sustain the unique cell-material interactions between the coral skeleton and hippocampal cells, with focus on two causative factors: 1) the 3D architecture and 2) the surface chemistry of the scaffold, i.e. - the ability of the cells to exploit the calcium of the scaffold biomaterial.

METHODS: Experiments were performed in the following steps: (i) underwater cloning and fabrication of microcolonies of the hydrozoan Millepora dichotoma, (ii) incubation and labeling with calcein and $^{45}$Ca$^{2+}$, (iii) seeding of neural cells from rat postnatal 1-2, (iv) calcium uptake measurements (calcein by fluorimetry and immunofluorescence microscopy, and $^{45}$Ca$^{2+}$ by radioactivity.

RESULTS: To check for aragonite-derived calcium incorporation by the cells we exposed cloned hydrozoan species Millepora dichotoma to calcein or $^{45}$Ca$^{2+}$ which were incorporated into the organism's growing skeleton, and thereby available for the cultured cells. We found that hippocampal cells took up aragonite-derived calcium, with the uptake being enhanced when the availability of extracellular calcium ions was reduced (chelated with EGTA). When the cells were cultured on coral skeletons that had been coated with gold, a mean to dissect out the role of the 3D surface architecture from its chemistry, cell survival was reduced but not arrested, suggesting a role for matrix 3D architecture in neural survival. Hence, the durability of cultures on coralline aragonite matrices is due both to its 3D porous surface and its function as a calcium nurturing scaffold.

DISCUSSION & CONCLUSIONS: Here we show that although, three dimensional architecture is an important factor for tissue growth and durability, the aragonite biomaterial itself is acting as an extremely bioactive vehicle by a unique way of providing the cells with calcium ions, enhancing neuronal survival. We posit that the translocation of calcium from the biomaterial to the cells activates a variety of membrane-bound signaling molecules, which affect the subsequent cell behavior. Such cell-material interactions hint at the potential of porous aragonite matrices in the fabrication of advanced biomaterials for neural tissue engineering applications.


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