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***In vitro* neural stem cell niche grown in 3D scaffold**

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Recent studies show that adult neural tissues can harbor stem cells within unique niches. In the mammalian central nervous system, neural stem cell (NSC) niches are present in the dentate gyrus and the subventricular zone (SVZ). Stem cells in the well-characterized SVZ exist in a microenvironment established by surrounding cells and tissue components including transit-amplifying cells, neuroblasts, ependymal cells, blood vessels and a basal lamina. Within this microenvironment, stem cell proliferation and differentiation are regulated. We have recently described a novel cell-attached NSC culture system, derived from mouse embryonic stem (ES) cells, that displays elements of a NSC niche in the absence of exogenously applied mitogens or complex physical scaffolding. In this study, we report our initial attempts to move this *in vitro* niche into a 3D scaffold, PuraMatrix. PuraMatrix is a peptide hydrogel developed by BD Biosciences that self-assembles into a complex molecular matrix in culture upon addition of media. This scaffold acts to anchor cultured cells in a tissue-like microenvironment. Here we report the addition of neuralized mouse ES cells into this scaffold in the presence of various basement membrane components. Mouse Laminin-1 alone, three concentrations of entactin/collagen/laminin (ECL), and culture media alone were tested with PuraMatrix to identify the optimal combination for development of the *in vitro* niche. Microscopic analysis of cell culture morphology revealed that the highest concentration of ECL produced the best substrate for niche growth and survival. The data demonstrate that cellular aggregation occurred in several of the experimental groups, but concentrated ECL led to the best process development and connectivity between adjacent cellular aggregates. 3D culture of a neuralized stem cell population may contribute to better understanding of the process of neurogenesis and NSC niche formation and regulation. In addition, our results may have implications for application of cells stem in cellular transplant therapeutics.