

Schaffer Webinar Questions

Thursday, January 21 at 2010, 2-3 PM EST

Has the phenotypic attributes of the cells that propagate with these polypeptides been characterized & compared with native cells

Neural stem cells have been passaged onto these surfaces for several months and we have characterized their behavior, proliferation rates, karyotype and differentiation and have never seen a functional difference. We don't think the surfaces are changing their properties or selecting for a certain type of cell. For hESCs we haven't characterized as broadly but still see no functional difference. Additionally, an embryoid body assay shows no difference in cell differentiation.

What is the enrichment of neurons vs. other cell types?

The protocol that we take the cell through is we do an embryoid differentiation to create a stochastic mixture of the three germ layers, we plate them under selective conditions to select for neural ectodermal cells using laminin surfaces and defined medium. That's the point where we pattern these cells to differentiation into one sub region vs. another of the central nervous system and when we add sonic hedgehog. So the resulting cultures are expanded and differentiated for several weeks in the presence of polyvalent sonic hedgehog have 80% beta-tubulin telling us they are neurons. We find that a large fraction of the beta-tubulin cells express markers of the dopaminergic lineage. That number is about 20-30% using the standard differentiation systems that have been reported in literature and we achieve upwards of 60% dopaminergic differentiation in the presence of polyvalent sonic hedgehog so this is a nice trajectory.

Can polyvalent Hedgehog complexes be combined with ECM proteins such as the peptide with the RGD sequence to create a potential synergy and further promote differentiation?

Yes, for example, the system used in the literature to produce neural precursors out of hESCs involves the EB stage and then plating cells on the laminin in the presence of Fgf2 and neural medium. That's a human protein purified from placental tissue which we want to move away from. We have generated synthetic surfaces with defined peptides by learning from adult neural stem cell work to create peptides that can support the viability of these human stem cell derived neural precursors and that is the direction we want to take: to be able to take a hESC into a fully defined neuron.

How about using a more "neutral" hydrogel and let mature cells produce the microenvironment that could lead to stem cell differentiation?

We asked the question of if we initially plate hESCs on Matrigel, does that substrate stay as Matrigel. In other words, do the cells themselves excrete matrix proteins to play a role in microenvironmental signaling?

The cells very actively secrete matrix proteins, almost all that we stain for. The cells were producing collagen 1, collagen 4, laminin, fibronectin. This indicates that the cells do have the potential to remodel their matrix environment with time.

Our current system does not enable them to do that. We have protein resistant layers using PEG so what we are doing is finding sum total of matrix input signals for self renewal. We could pull off PEG and cross link ligands onto nylon or polyacrylamide to make these surfaces more permissive for protein adsorption which would allow cells to lay down their own matrix. This could allow the cells to teach us something about what matrix signals they require.

Are there studies (in your lab or otherwise) that show that replacing neurons in hippocampus restores function?

There are definitely loss of function studies – animal models using CRE-LOX allows you to inducibly kill or reduce activity of neural stem cells within the adult hippocampus and these animals suffer deficits in hippocampal dependent learning tasks. So all of the activities we rely on the hippocampus for such as spatial memories, the ability to package individual memories for long term storage in the cortex, all of these functions have the potential to be compromised by reduced levels of neurogenesis.

To my knowledge the opposite experiment has not been done where you upregulate neurogenesis and increase at steady-state the number of neurons in the hippocampus. Are these animals any “smarter”?

We are also interested in these for cell replacement therapies where you could assist the hippocampus in recovering from Alzheimer’s disease for instance.

Are you aware of efforts to develop mathematical models for these systems?

We have had 3 publications over the years in mathematical analysis of signaling systems and there are other terrific investigators working on this including Peter Zandstra at U Toronto who is prominent among them. We have several publications, one in Biophysical Journal, one in Development and most recently in PLOS computational biology in which we have modeled how intercellular networks are activated by and process signaling info and basically take a signal from the microenvironment and enable the cell to make a downstream fate choice in response to these signals. Fate choices are typically all or nothing decisions so binary or trinary and the inputs are analog so there needs to basically be an analog to binary conversion and we have been studying the process by which signal transduction pathways allow the cell to make these critical decisions.