Microenvironment Dimensionality and Cells of the Nervous System: Significant Effects on Cell Shape and Signaling

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Abstract: Translating information from two-dimensional (2D) in vitro experiments into three-dimensional (3D) systems has been a major hurdle in the use of biopolymers for tissue repair. In order to design improved culture environments and responsive architectures for neuronal repair, our goal is to advance the understanding of how cells of the nervous system respond to 3D environments. We show that a 3D culture platform invokes characteristic neuronal and glial morphologies, overcoming the loss of these essential cell responses that occurs in 2D culture. We show that integrin signaling as well as extracellular matrix (ECM) synthesis are drastically altered in 3D vs 2D with 3D more closely mimicking the in vivo scenario. These findings have been integrated into the design of synthetic hydrogel systems with defined cell-scaffold, solute-scaffold and degradation properties. We further show that the synthetic gels can be used to direct neural progenitor cell (NPC) differentiation for ultimate application towards transplant into sites of neuronal injury or degeneration.

Methods: Dorsal root ganglia (DRG) neurons were dissected from E13.5-14.5 mice [1] and gray matter astrocytes were isolated from P1-3 mouse cerebral cortices. The cells were cultured on 2D collagen-coated glass and in 3D collagen gels for 2-10 d. Fixed samples were analyzed by immunofluorescence and imaged by confocal microscopy. Neuronal morphologies were quantified with Neurolucida [1]. Astrocytes were scored by morphology: 1) round, 2) bipolar, 3) stellate resembling mature astrocytes in vivo, and 4) multipolar. Hydrolytically degradable polyethylene glycol (PEG) gels were synthesized and characterized for degradation rate, solute-scaffold and cell-scaffold interactions according to published methods [2] Millipore ReNcell-CX (human neural progenitor) were cultured in PEG gels for up to 15 d. Degradation time [2] and cell fate marker expression were determined. Statistical analysis was performed with Analyse-it software via t-test, ANOVA or Mann-Whitney ($p<0.05$).

Results and Discussion: For DRG neurons, we show that β1-integrin signaling underlies the cells ability to sense the 3D environment and elaborate the in vivo cell morphology. β1-integrin, FAK, FAKpY397 and FAKpY861 were present in point contacts throughout neurons in 2D. In 3D, however, β1-integrin, FAK and FAKpY861 reactivity were diffuse and FAKpY397 was significantly reduced vs 2D (**Fig 1a**). These findings agree with previous studies of altered cell-matrix adhesions and FAKpY397 expression in 3D and support the idea that the 3D milieu results in decreased β1-integrin and altered FAK signaling. Treatment with HMβ1-1, a function-blocking β1-integrin antibody, did not alter point contact formation of neurons in 2D, but resulted in shorter neurites in 2D and 3D (**Fig 1b**). In 2D, neurons were multipolar with unbranched neurites regardless of integrin inhibition. In 3D with no treatment, neurons were unipolar with branched neurites, as in vivo. In 3D with integrin inhibition, neurons appeared as in 2D.

For astrocytes, a 3D culture environment allows astrocytes to survive and transition from a population of round and bipolar cells at 4 d towards stellate and multipolar cells at 10 d; in 2D, however, this population shift was lost by 4 d and yielded no stellate or multipolar cells (**Fig 1c-d**). At 10 d, the distribution of astrocyte morphology groups were not affected by cell density, position in the 3D gel or the presence of serum; but significantly altered in 3D vs 2D culture. Further, the expression and deposition patterns of ECM are altered in 2D vs 3D culture with 3D providing a ECM milieu that mimics the healthy cerebral cortical whereas 2D culture more closely resembles the ECM of glial scar tissue. We suggest that cell microenvironment dimensionality influences astrocyte morphology with 3D more closely mimicking a key aspect of the in vivo scenario that may uniquely allow a transition from newly born astrocyte to fully differentiated stellate morphology.

For NPCs in PEG gels, we show that encapsulated cells are largely undifferentiated (nestin+) and associated with altered gel properties (greater stiffness, slower degradation) vs acellular gels, possibly due to ECM synthesis. NPCs released from the degraded PEG gels had significantly more neurons and less astrocytes vs 2D culture (**Fig 1e**).

Conclusions: 3D culture allows neurons and glia to elaborate the in vivo morphology, providing a more suitable milieu to mimic the in vivo scenario vs 2D. Further, synthetic gels with defined interactions between the scaffold and cells/solutes can direct NPCs towards the neuronal fate.

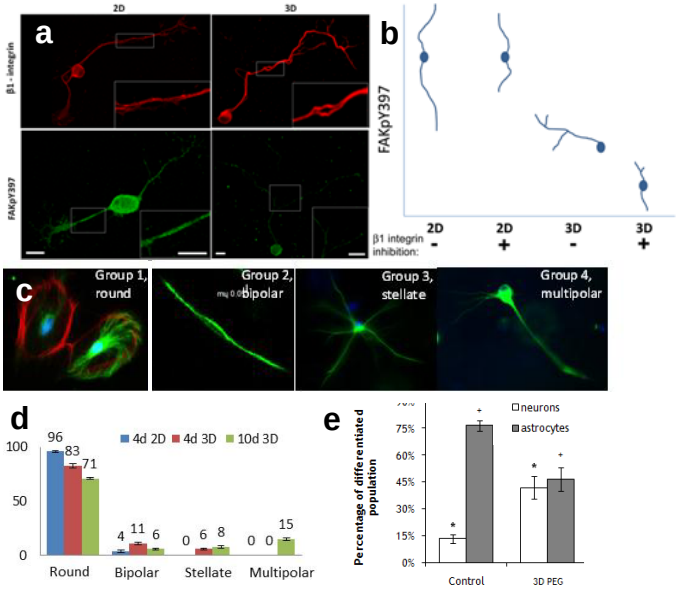

Fig 1: a: DRG β1-integrin and FAKpY397 expression in 2D and 3D. **b:** Schematic of DRG morphology in 2D vs 3D with β1-integrin inhibition. **c:** Astrocytes cultured in 2D and 3D were scored by morphology (GFAP, green; actin, red; nuclei, blue). **d:** 3D culture at 10 d allowed the most stellate cells and multipolar cells. **e.** 3D PEG gels increase NPC differentiation to the neuronal phenotype following gel release.

References: [1] Ribeiro A. Tissue Eng. 2012, 18: 93; Neurosci. 2013, 248:67; [2] Zustiak S. Biotech Prog 2013, 29:1255; Soft Matter 2010, 6:3609; Biomacromol 2010, 11:1348