Oligodendrocyte Survival, Proliferation, and Intracellular Redox State is Dependent on 3D Hydrogel Mechanics and Degradation

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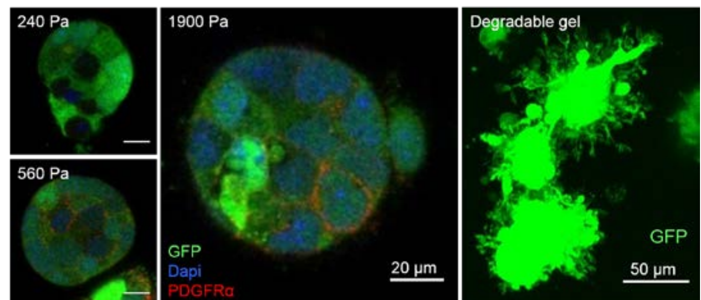
Introduction: Millions of people suffer from damage or disease to the central nervous system (CNS), such as multiple sclerosis, resulting in a loss of myelin. In the CNS, oligodendrocytes extend processes which wrap around neuronal axons to generate myelin, an electrically insulating layer conducive to rapid, controlled neuronal signaling. Diminished myelin distorts and slows neuronal communication and causes neuronal degeneration and death. Cell loaded biomaterials are a potential method of repair, but have rarely been investigated for oligodendroglial specification[1]. Here, we investigate how mechanical properties of a polyethylene glycol based hydrogel affect proliferation of two oligodendrocyte precursor cell (OPC) lines.

Materials and Methods: Hydrogels with controllable storage moduli were formed by photoinitiation of methacrylated polyethylene glycol (PEG) or PEG-poly(lactic acid) (PEG-PLA) with molecular weights and concentrations ranging from 4600 to 8000 g mol^{-1} and 6% to 20% (wt/v), respectively. Gelation was accomplished by photoinitiation of LAP at 365 nm and 4 mW/cm^2 with OPCs[2-3] encapsulated at $1x10^7$ cells/ml. ATP and DNA were quantitatively analyzed at discrete time points using high throughput luminescence and fluorescence assays to determine the viability and proliferation. LIVE/DEAD confocal microscopy verified ATP and DNA results and immunocytochemical staining revealed the presence and maintenence of OPC lineages.

Results and Discussion: PEG-dimethacrylate hydrogels with storage moduli from 230 to 1000 Pa were formed by tuning the concentration and molecular weight from 6% to 10% (wt/v) and 4600 to 8000 Da, respectively. When cells were encapsulated in the hydrogels, they proliferated in a stiffness dependent trend, where the largest increases in ATP and DNA concentrations were found in the most compliant hydrogel formulation. In gels with storage moduli of 230 Pa, the concentration of ATP was found to increase 12 fold over seven days while DNA increased 44% over seven days. To test the influence of antioxidants, we incorporated lactic acid into the hydrogel both as a soluble factor or into the polymer backbone where it can be released through hydrolytic degradation. As a measure of intracellular redox state, glutathione content within cells was measured in its reduced GSH form and in its oxidized GSSG disulfide form. Results from determining the ratio of reduced GSH to total glutathione (GSH and GSSG) suggest that incorporating lactic acid further reduces the intracellular redox state and increases cellular proliferation. These findings suggest the potential use of tunable PEG hydrogel systems to promote OPC growth, increase oligodendrocyte maturation, and repair the myelin sheath.



Cells differentiate according to material stiffness. More cells are PDGFRα+ in stiffer hydrogels indicating a more mature OPC or oligodendrocyte lineage. In degradable gels, live imaging shows OPCs lose the rounded spheroid shape and extend processes, beginning to adopt oligodendrocyte morphology.

Conclusions: The material properties of PEG hydrogels influence oligodendrocyte-like cell proliferation, indicating their potential use in controlled growth of oligodendrocytes for CNS regeneration. In particular, these cell lines proliferate more the more compliant matrices but differentiate more in stiffer gels. Currently, the cellular phenotype is being investigated, from gene expression to redox state, to further the potential use for oligodendrocyte regeneration. Additional research is incorporating degradable subunits into the hydrogel to promote a favorable intracellular redox state.

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References: [1]Straley, KS et al., *J. Neurotrauma* 2010, 23, 1-19. [2]Liu, C. et al., *Cell* 2011, 146, 209-221. [3]Verity, AN et al., *Journal of Neurochemistry* 1993, 60, 577-587.