Electrospun Fiber-Glia White Matter Mimetics

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Statement of Purpose: Glioblastoma multiforme (GBM) is a malignant tumor of the brain characterized by resistance to chemotherapy, radio-resistant cell populations, and high infiltrative potential [1-2]. Despite decades of *in vitro* testing and molecular analyses, the migration behavior of these tumors remains poorly understood and the median survival for patients remains 12-15 months [3]. Little progress has been made in treatment due to our inability to target migrating cells *in vivo* and an absence of relevant *in vitro* models. GBMs are known to preferentially migrate along white matter tracks in the brain [3]. To simulate these white matter tracks *in vitro* we have cultured and differentiated rat glial precursor cells (GPCs) on aligned electrospun poly(□-caprolactone) (PCL) fibers. Previous studies have used electrospun fibers to promote nerve regeneration and have demonstrated interactions between Schwann cells, fiber orientation, and myelination [4-5]. However, no studies on the myelination of synthetic fibers or oligodendrocyte/fiber interactions have been reported. At approximately two days after differentiation, GPCs start producing processes and lamella that align with fibers and stain positively for myelin basic protein (MBP). The myelinated fibers resemble myelinated axons in white matter and may be used in migration and myelination assays for future glioma and multiple sclerosis research. We report the effects of GPC cell density and co-culture with GBMs on the myelination of synthetic fibers.

Methods: Aligned PCL nanofibers were obtained from Nanofiber Solutions, LLC as 24 well inserts that fit directly into multiwell plates. The fiber diameters were between 200-1000 nm. The spacing between parallel fibers was less than the ~20 micron diameter of GPCs, preventing GPC adhesion to the underlying substrate. Disks and fibers were sterilized by soaking in ethanol for 20 minutes and air drying under a UV lamp. After sterilization, disks were washed three times with PBS and soaked in medium for 1 hour prior to seeding cells. GPCs (GIBCO) suspended in complete GPC medium (GIBCO) were seeded in monoculture with varying cell densities and in coculture with GBMs. Two days after seeding, complete GPC medium was replaced with differentiation medium lacking PDGF-AA, EGF, and bFGF but supplemented with 2% FBS. After 2 or 7 days of culture in differentiation medium, the samples were fixed for 20 minutes with 4% paraformaldehyde solution and blocked with Triton for 40 minutes. Primary antibodies were added and samples were refrigerated for 48 hours. Secondary antibodies were added and samples were incubated at 37 ºC for 40 minutes. Samples were washed with PBS between each of the fixation and staining steps. Samples for immunofluorescence imaging were stained for MBP, indicating myelination by oligodendrocytes (Millipore); and DAPI nuclear staining. Immunofluorescence confirmed the presence of myelin and allowed quantification of myelin producing cells and myelin coverage. Scanning electron micrographs (SEM) revealed the morphology of the myelin structures and their relationship with the fibers.

Results: Immunofluorescence images confirm differentiation of GPCs to the oligodendrocyte phenotype, myelination of synthetic fibers and alignment of cellular extensions. SEM micrographs confirm intimate interactions and adhesions between myelin producing cells and fibers (Fig. 1). Immunofluorescence analysis indicates an increase in myelination at higher GPC seeding densities as well as an increase in myelination when cocultured with the OSU2 cell line, a GBM line derived from a primary tumor and cultured with serum.

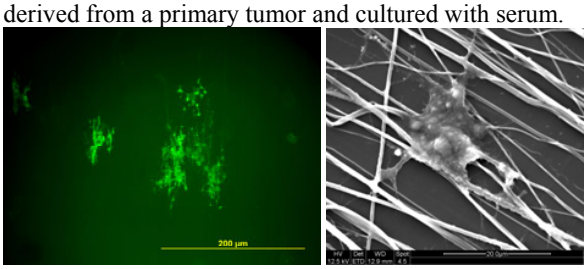

Figure 1. MBP stained GPCs on aligned PCL fibers (left). SEM of differentiated GPC on aligned PCL fibers (right).

Conclusions: Under the correct conditions, GPCs can be cultured to produce myelin on synthetic fibers. The myelinated fibers resemble those of myelinated axons in white matter and may provide a platform for the exploration of GBM and glial interactions. Although myelinated PCL fibers do not replicate intimate axon/oligodendrocyte interactions, they do present a simplified environment for the study of GBM migration along white matter tracks. Currently we are costaining GPC cultures for expression of MBP and glial fibrillary acidic protein (Millipore) to investigate the effects of culture conditions on cell differentiation. Also, we are examining MBP expression in combination with SEM to further understand myelin/fiber interactions. Future studies should investigate GBM migration mechanisms and rates along myelinated fibers as well as the potential of this platform as a myelination or, in the case of multiple sclerosis, demyelination study.

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