Enhancement of Astrocyte Migration Through Collagen-Genipin Gels in Response to Fibroblast Growth Factor-2

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Statement of Purpose: Astrocytes can play dual roles in the response to spinal cord injury (SCI) acting as both an inhibitory barrier and a trophic support for growth axons [1]. Therefore, migration of these cells into the defect as opposed to forming a scar at the periphery, may promote axon regeneration through the lesion. However, infiltration requires the conformal filling of the cyst-like lesion, which often forms after SCI, with a biomaterial scaffold encouraging of astrocyte migration. For this application, we investigated injectable collagen-based hydrogels covalently cross-linked with genipin [2] and incorporating fibroblast growth factor-2 (FGF-2) encapsulated within lipid microtubules (LMTs) for sustained delivery. FGF-2 is a potent growth factor released by multiple types of cells after injury to the CNS, which may contribute to the limitation of injury and induction of progenitor cells after SCI [3]. Additionally, FGF-2 accelerates *in vitro* models of astrocyte wound closure by attracting astrocytes into the defect [4].

Methods: Soluble type I rat-tail collagen (BD Biosciences, Franklin Lakes, NJ, USA) was diluted to 2 mg/ml and brought to neutral pH with NaOH and phosphate buffered saline, and genipin (Wako Pure Chemical, Japan) was added at 0.25 mM. Thermal gelation of the collagen solutions was induced by warming to 37 °C in an incubator. LMTs were prepared by thermal self-assembly using 1,2-bis-(triscosa-10,12-diynoyl)-sn-glycero-3-phosphocholine (DC8,9PC) (Avanti Polar lipids, Alabaster, AL, USA). LMTs were loaded overnight with 0.11 (low) or 1 mg/ml (high) recombinant human FGF-2 (Peprotec, Rocky Hill, NJ, USA), rinsed, and added to the gels at 0.1 mg/ml. Cortical astrocytes were harvested from post-natal day 2 Sprague Dawley rats and were seeded at 1 million cells/ml. An outgrowth migration assay (Fig 1) was used to evaluate *in vitro*: the number of primary rat astrocytes migrating from a core astrocyte-seeded collagen gel into the experimental collagen-genipin gels; and the distance to which they migrated. At day 10, gels were stained with calcein AM and visualized at 4x in an epifluorescence microscope.

Results: The presence of FGF-2 within the encapsulating gel significantly increased the number of astrocytes within the gel, their penetration distance into the gel, and caused them to move out in a chain migration pattern, compared to control gels without FGF-2 (Fig 2,3). Genipin cross-linking of the collagen gel decreased the number of migrating astrocytes, compared to the non-cross-linked gels; however, incorporation of FGF-2-containing LMTs within genipin-cross-linked gels restored the astrocyte migration to levels approaching non-cross-linked gels incorporating FGF-2. With increasing concentrations of FGF-2, the cells became more clustered as they migrated from the core gel, forming disorganized aggregates in addition to directed projections.

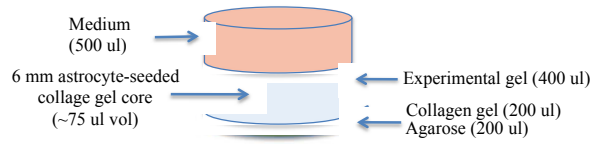

Figure 1. Migration assay setup consisting of a core astrocyte seeded collagen gel encapsulated within a collagen gel containing the experimental factors (genipin, LMTs, FGF-2 low: ~20ng/ml (low) high: ~200 ng/ml).

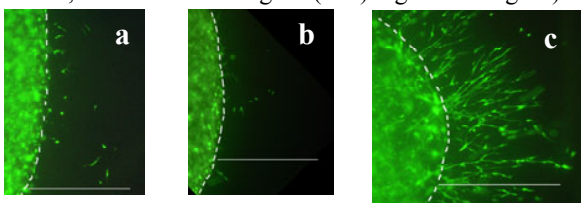

Figure 2. Fluorescent micrographs of calcein AM stained astrocytes in the migration assay a. Col b. Col-Gen c. Col-Gen-LMT FGF low. Scale bar 1 mm.

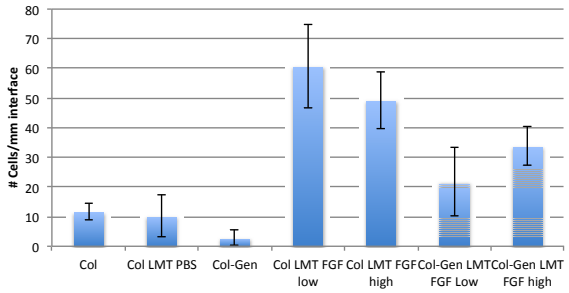

Figure 3. **:** Number of cells migrating into the encapsulating collagen gel normalized by the total length (in mm) of gel interface imaged. Mean ± st dev. n= 4-6.

Conclusions: Overall, injectable collagen-genipin hydrogels containing FGF-2-containing LMTs are a promising candidate for the treatment for SCI through the attraction of astrocytes into the gel-filled defect. The addition of genipin to the gels is inhibitory to migration unless FGF-2 is present. While genipin itself has low cytotoxicity especially at the concentrations examined, it still can interfere with cellular motility and proliferation. Importantly, the addition of FGF-2 to the gels especially when incorporated into LMTs can mitigate these negative effects while still maintaining the required increases in strength and degradation resistance imparted by genipin to allow for appropriate remodeling of the gel.

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