Injectable Gelatin-Hydroxyphenylpropionic Hydrogels with Tunable Crosslinking for Supporting and Influencing Adult Neural Progenitor Cells

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Statement of Purpose: Many brain injuries, such as stroke, often result in cavitary defects that lose structural support for transplanted multipotent neural progenitor cells (NPCs) or endogenous NPCs arising from specific regions in the brain post-injury. Biomaterials can serve as provisional matrices in the defects to support proliferation, migration and differentiation of NPCs and enable them to mediate regenerative responses. Here, we investigate how a novel injectable gelatin hydroxyphenylpropionic acid (Gtn-HPA) hydrogel, which features independent tuning of mechanical properties and gelation rate,[1] supports and influences proliferation, migration and differentiation of adult NPCs (aNPCs). This work will serve to evaluate Gtn-HPA as a potential biomaterial to support transplanted or endogenous NPCs.

Methods: aNPCs were previously isolated from the hippocampus of adult female Fischer 344 rats (F. Gage, Salk Institute). Gtn-HPA, which could be crosslinked by horseradish peroxidase and H_2O_2 via an enzymatic-mediated oxidative reaction, was synthesized as described previously[1] (M. Kurisawa, Institute of Bioengineering and Nanotechnology, Singapore). Hydrogels were crosslinked with 850, 1000, 1200 and 1700μM H_2O_2 (hereafter referred to as G-850, G-1000, G-1200 and G-1700 respectively). Rheological measurements were performed at a constant strain of 1% and frequency of 1Hz. Adult NPCs were either plated as monolayer culture on poly-ornithine/laminin (P-Orn/Lam) -coated chambers slides or encapsulated within Gtn-HPA gels. Proliferation of adult NPCs was induced by 10ng/ml FGF-2 and measured at day 4 and 7 using a DNA picogreen assay. To induce mixed differentiation, aNPCs were treated with 1μM retinoic acid (RA) and 1% fetal bovine serum (FBS) for 6 days. At day 6 post-differentiation, processes extending from neurospheres were visualized by phase-contrast microscopy and analyzed with ImageJ software. Immunostaining for neuronal marker (beta tubulin III TuJ1) and astrocytic marker (glial fibrillary acidic protein GFAP) was performed on the chamber slides and three 50μm cryosections for each hydrogel by using mouse anti-TuJ1 (1:5000, Covance), rabbit anti-GFAP (1:5000, Dako) and respective secondary antibodies (1:200, Jackson Immunoresearch). Analysis of variance (ANOVA) followed by Fisher's post-hoc test was performed using StatView software.

Results: Varying degrees of crosslinking were found to produce hydrogels with storage modulus (G') ranging from 449 Pa, which approximates that of adult rat brain,[2] to 1717 Pa (Fig. 1). At all degrees of crosslinking, Gtn-HPA hydrogels supported proliferation of aNPCs, albeit at a lower level compared to monolayer culture on P-Orn/Lam surfaces (Fig. 2). One-factor ANOVA revealed a significant effect of crosslinking degree on proliferation of aNPCs ($p<0.05$). After 6 days of treatment with RA and FBS, aNPCs cultured within G-1000, G-1200 and G-1700 hydrogels were observed to form neurospheres with processes extending outwards. Processes were significantly longer in G-1000 and G-1200 (~120μm) compared to G-1700 (~70μm). Immunostaining for TuJ1 further revealed that processes in G-1700 were only neurites while those in G-1000 and G-1200 included migratory chains of neurons (Fig. 3). Gtn-HPA hydrogels also allowed for neuronal and astrocytic differentiation of aNPCs (Fig. 4). Notably, aNPCs cultured within Gtn-HPA hydrogels preferentially differentiated into neurons when compared to monolayer culture on P-Orn/ Lam surfaces. However, the proportion of aNPCs committed to neuronal or astrocytic lineages was not affected by the degree of crosslinking.

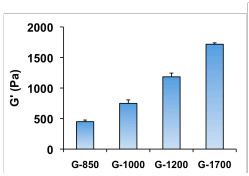

Fig. 1: Storage modulus (G') of Gtn-HPA hydrogels (n=3)

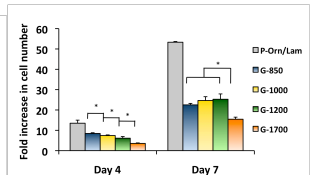

Fig. 2: Proliferation of aNPCs in Gtn-HPA hydrogels (n=4, * $p<0.05$)

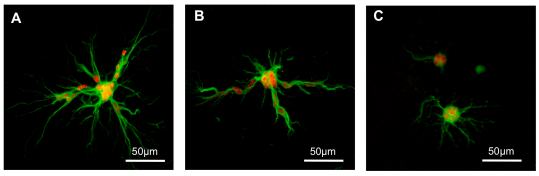

Fig. 3: Neurospheres extending migratory chains in (A) G-1000 and (B) G-1200 hydrogels but only neurites in (C) G-1700 hydrogels (TuJ1 – Green, DAPI – Red)

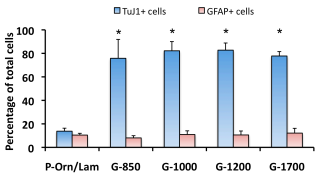

Fig. 4: Differentiation of aNPCs on P-Orn/Lam surfaces and within Gtn-HPA hydrogels (n=4, * $p<0.001$ compared to P-Orn/Lam)

Conclusions: Injectable Gtn-HPA hydrogels provide a permissive environment for proliferation, migration and differentiation of aNPCs, commending them for further *in vivo* studies. The degree of crosslinking influences the rate of proliferation and chain migration but not differentiation. Gtn-HPA results in a higher proportion of neurons after mixed differentiation compared to monolayer culture. It is of interest to investigate if Gtn-HPA actively instructs neuronal differentiation and/or selects for survival and proliferation of neuronal progenitors.

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References:

1. Wang LS. Biomaterials 2010;31:1148-1157
2. Georges PC. Biophys. J. 2006;90:3012-3018