Stimulation of Neurite Outgrowth Using a Positively Charged Hydrogel

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Introduction: The current clinical approach to repair peripheral nerve damage involves utilization of an autologous nerve graft. As an alternative to nerve autografts, natural and synthetic tubular nerve guidance channels to bridge the gap between severed nerve ends are being extensively explored. Past works have demonstrated that electrical charges play an important role in stimulating either the proliferation or differentiation of nerve cells.[1] In this work, photocrosslinkable oligo-(polyethylene glycol) fumarate (OPF) was copolymerized with [2-(methacryloyloxy) ethyl]-trimethyl ammonium chloride (MAETAC) to produce a positively charged hydrogel, as a substrate to enhance nerve cell differentiation. We demonstrated that incorporation of positive charge in hydrogel promotes neurite outgrowth of dissociated dorsal root ganglion (DRG) cells and DRG explants. Explant neurite outgrowth on the charged hydrogels was evaluated in the presence and absence of laminin derived- peptide.

Materials and Methods: OPF was synthesized according to a previously published method.[2] OPF macromer (1gr) was dissolved in deionized water containing 0.05% (w/w) of a photoinitiator (Irgacure 2959, Ciba-Specialty Chemicals) and 0.3 gr N-vinyl pyrrolidinone. To fabricate hydrogel with 10% and 20% charge, MAETAC (75%, Aldrich) was added to the solution at the concentrations of 200 and 400 mM, respectively. The mixture was pipetted between glass slides with a 1 mm spacer and polymerized using UV light (365 nm) at an intensity of ~8mW/cm^2 (Blak-Ray Model 100AP) for 30 min.

Neuronal cultures: DRGs were excised from E15 Sprague-Dawley rat pups (Harlam) trypsinized and mechanically dissociated; the cell suspension thus obtained was plated onto the hydrogels with different charge in MEM, (minimum essential medium, GIBCO) supplemented with 15% calf serum in dissociated or 10% calf serum for DRG explant cultures, nerve growth factor (NGF, 8ng/ml), glucose (0.6% w: v), and L glutamine (1.4 μM; Sigma). For dissociated cultures the DRG were treated with 0.25% trypsin in hanks balanced salts for 30 minutes at 37^{o}C and disrupted through a restricted glass pipette. The DRGs were then plated on collagen coated plates. Contaminating non-neuronal supporting cells were eliminated by treatment with 4μM 5 Fluoro-2-deoxy-uridine (FUDR)/ 4μM Uridine (Sigma, St. Louis, MO) which was added to the media and incubated in a humidified incubator at 37^{o}C, 5% CO_2 for 3 to 5 days.

DRG explants: DRGs were dissected from E15 rat embryos and plated onto the test OPF hydrogel discs. 10-15 DRGs explants were cultured on each hydrogels disk with different charge and in vitro analysis and quantification of neurite extension on charged modified hydrogels were performed after 24 and 40 hours using a digital image analysis system of a Zeiss Axiovert 35 with a Nikon CCD camera. Light microscope images of the DRGs in culture were captured and eight longest neurites were traced and their lengths measured using Image J software obtained from NIH.

Results: Dissociated DRG cells were seeded onto the hydrogels with different charge density. Cells attached to a greater extent on the hydrogels with 10% and 20% charged, while only few cells were observed on the hydrogels without charge. As shown in Figure 1, DRG cells extended their neurites more readily on the surface of positively charged hydrogels than unmodified hydrogels.

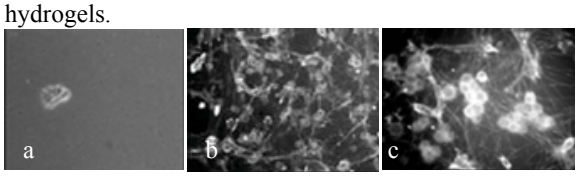

Figure 1: Optical micrographs of dissociated DRG cells on (a) unmodified hydrogel, (b) positively charged hydrogel at low magnification, (c) and higher magnification taken at 40X lens after 7 days.

Figure 2 shows quantification of the neurite outgrowth from DRG explants on hydrogels with different charge levels in comparison to the hydrogel without charge and laminin coated plastic as control. These data showed incorporation of the positively charged monomer into the hydrogels improved neurite outgrowth from DRG explants significantly ($p<0.05$) in the presence and absence of laminin derived peptide.

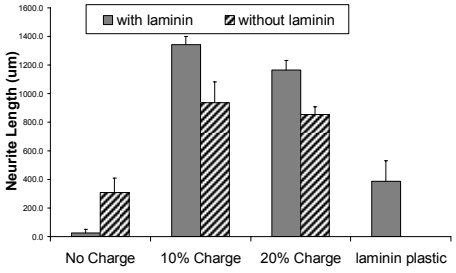

Figure 2: Neurite outgrowth from DRG explants on various substrates.

Conclusions: Our results showed modification of photocrosslinkable OPF with positively charged monomer improved nerve cells attachment and neurite outgrowth. This material is a candidate scaffold for nerve tissue engineering.

References:

1-Schmidt CE. PNAS 1997; 94: 8948-8953.
2- Jo S. Macromolecules 2001; 34:2839-2844.

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